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A MOSQUITO NET FOR USE IN THE PHILIPPINE ISLANDS

EXPERIMENTAL STUDIES AND CANVASS OF MATERIALS [1]

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TEN PLATES AND ONE TEXT FIGURE

INTRODUCTION

Mosquito nets, sometimes called mosquito bars, bed nets, tents, or *mosquiteros,* have been used since very early times to protect

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man from the bites of mosquitoes. Von Kühlewein(1) and Hehir,(2) for instance, stated that Herodotus noted that several hundred years before Christ Egyptians had nets which were used in the daytime for fishing and in the nighttime as a protection against mosquitoes.

Schroeder(3) stated that Marco Polo in the thirteenth century in a description of a temple on the Coromandel Coast of India mentioned beds with curtains which could be closed by a purse string to keep out biting flies and other vermin.

Ross(4) stated that *conopeum,* or mosquito netting, sheltered wealthy Roman babies as they slept, and that such bed nets were alluded to by Herodotus, Varro, Horace, Propertius, Juvenal, and Paulus Silentiarius.

In the Philippines it would appear that *"mosquiteros"* were used by the Spaniards soon after they came to the Islands.

Blair and Robertson,(5) presenting a translation of Aduarte's Historia of 1640, include the following statement about the martyrdom of Father Fray Guillermo Cortet:

> The Province directed him to teach theology in the college of Sancto Thomas at Manila, which he did obediently, putting aside his desire to go to Japon. That he might have more time and ease in the holy exercise of prayer, he never undressed at night during the last twenty years of his life, but slept seated in a chair. This country is infested with multitudes of annoying mosquitoes; but he did not take advantage of the common means of preventing them, which is a tent, something permitted to all the religious. He would not accept one, but offered to the Lord the stings of the gnats, which is no small mortification and penance.

We have not been able to find any definite references to the use of such nets prior to the Spanish occupation. But Dr. H. O. Beyer,(6) and also Minier(7) and Miller,(8) stated that sinamay netting was woven in the Philippines before the Spaniards came. There are very early references by Chinese authors to this transparent cloth. Doctor Beyer(6) also noted that a net is used at one stage in typical Moro weddings. It therefore seems not unlikely that nets to ward off insects were used locally in very early times.

As to the usefulness of mosquito nets in the Philippines there is little doubt. In common with all tropical countries, these Islands have an abundant mosquito fauna. There are some twenty-seven species of *Anopheles,* several species of *Aëdes,* and some hundreds of species of *Culex.* Naturally, mosquito-borne diseases also abound. Malaria is known to be carried by *Anopheles minimus* var. *flavirostris,* and perhaps also by *A. filipinæ, A.*

maculatus, and *A. mangyanus.* Dengue is known to be carried by *Aëdes ægypti* and *Aëdes albopictus.* Filariasis is found to a limited extent and is probably carried by *Culex* species. Yellow fever has never occurred in the Philippines. *Culicoides* species are found in the Philippines, and sometimes bite annoyingly, but they are so small that no ordinary netting will keep them out. We do not recommend that nets be fine enough to exclude *Culicoides,* except under very special conditions. The standard net presented in this paper will not keep out these minute flies.

It is in prophylaxis against malaria that mosquito nets have the greatest potential value in the Philippines. Malarial fevers are found throughout the length and breadth of the Archipelago wherever there are fresh running streams. Practically all foothill areas are malarious. Exactly what the incidence of this disease is cannot be determined, because a very high percentage of illness in the provinces does not receive medical attention. It is the rule rather than the exception that cause of death, outside the populous centers, is diagnosed by laymen for the official reports, because physicians are not available. However, one of us(9) has recently ventured the opinion, based on all available evidence, that there are probably some 2,000,000 cases of malaria with more than 10,000 deaths yearly in the Philippines.

It seems likely that in this country the most important weapon against malaria is the mosquito net. There is practically nothing which the average citizen can do to combat this disease except to use a protecting net at night. Larva control is important but cannot be carried out by individual householders. It must be accomplished by community effort supervised by the Bureau of Health. Antimalaria drugs are essential but are so expensive that few can afford to take them in curative amounts.

To one unacquainted with this country the question might occur as to why it would not be better to advise the screening of houses or bedrooms rather than the use of bed nets. The answer becomes obvious when it is realized that, in common with many tropical countries of the Far East, most rural houses are made of woven materials loosely put together, with open eaves and with bamboo floors having wide cracks between the strips. In the Philippines a majority of rural habitations are made with woven strips of nipa palm leaf for walls, with floors of split bamboo laid in strips, 1 or 2 centimeters apart. Roofs are made of nipa palm, anajao palm, or cogon grass, occasionally of galvanized iron. The rooms have no ceilings and it is usual to see wide spaces between the top of the walls and the over-

hanging roof and around the windows and doors. These houses are usually on posts, 2 or 3 meters above the ground. They are light, dry, and airy, and are therefore not attractive to mosquitoes in the daytime.

It is not feasible to screen rural houses so constructed. In each town there are houses built of strong materials in such a way as to make screening not only practical but preferable to bed nets. But by far the greater number of rural houses could not be mosquito-proofed at any reasonable cost. Moreover, even if screening were feasible it would not be tolerated during the daytime when it lessens comfort and has little anti-mosquito value.

There is no reason, theoretically, why every person living in a malarious region should not use a mosquito net. It is true that nets decrease slightly the circulation of air, but on the other hand they afford excellent assistance in malaria prophylaxis and offer welcome protection from myriads of insects. They can be made for a price within the means of the people.

Actually there are several practical reasons why nets are not widely used in malarious places. Suitable nets are usually not easily available. There is also an inertia of the masses to new devices not having immediate, obvious, and overwhelming advantages. This inertia is resistant beyond belief. Every artifice at the command of health officers must be used to overcome it.

In the case of mosquito nets it is essential that standard, cheap, effective nets be made available locally so that the householder, in supplying himself with a net, will be put to only the very minimum of effort. It will require active, incessant, house-to-house propaganda by health inspectors and, more especially, by health nurses.

The first reference to the use of mosquito nets in malaria prophylaxis in the Philippines is a circular by Lippincott reported by Love(26) as having been included in the Surgeon General's report from 1898. Lippincott advised the Army as follows: "Mosquitoes have been accused of causing malaria, and whether it is true or not, it is wise to protect ourselves from them. Mosquito netting is desirable." The United States Army reports thereafter repeatedly emphasize the importance of mosquito nets. In 1907, Vedder(10) devised a new net suitable for field use by the soldiers. Although the Army nets at first were not satisfactory, one of us(9) has expressed the opinion that, subsequent to 1906, nets appear to have reduced the incidence of

malaria in the Army by about 75 per cent. The use of these nets was strictly enforced. Page,(11) for example, noted that at the Lucena barracks men were even court-martialed for not using their mosquito nets properly.

Chamberlain, Vedder, and Barber(12) tested bronze mosquito netting against the passage of mosquitoes, experimenting with 8-, 12-, 14-, and 16-mesh, trying both vertical and horizontal partitions. Practical field tests were also carried out. It was found that 16-mesh bronze gauze was impervious to *"Myzomyia rossii," "Myzorhynchus barbirostris,"* and *"Culex fatigans,"* but *"Stegomyia calopus"* passed through the 16-mesh wire gauze.

Manalang(13) reported that according to his observations *"A. minimus"* seemed to feed on humans chiefly between 10 p. m. and 2 a. m. He also noted that the wheal caused by its bite disappeared in one or two hours. These reports explain why infections may occur in the Philippines when the victims stoutly maintain that there were no mosquitoes biting them. The *minimus* adult is unobtrusive and spreads her sporozoites apparently late at night without attracting the attention of the householder. In this paper Manalang(13) also expressed the opinion that local mosquito nets require not less than sixteen meshes per linear inch.

In 1923 Tiedeman(14) reported that a survey of 1,600 persons revealed only 12.4 per cent using mosquito bars of any kind. He wrote, "the survey revealed that practically no efforts are being made by the people to protect themselves from the bites of mosquitoes. Mosquito bars are not in general use."

Recent mosquito-net surveys would seem to indicate that the use of such protection is becoming more general. For example, the following reports from Aritao, Nueva Vizcaya, and Calauan, Laguna, seem to indicate this.

Mosquito-net survey, Aritao, Nueva Vizcaya Province, Luzon, 1933.

Total houses	143
Said to use nets	94
No nets in house	49
Parasite index, 299 persons examined	31.8
Splenic index, 31 examinations	33.3

Mosquito-net survey, Calauan, Laguna Province, Luzon, 1933.

Total population canvassed (poblacion)	1,110
Number of mosquito nets [a]	407
Parasite index, 76 school children	28.9
Splenic index, 76 school children	40.8

[a] Several persons may sleep under one net; 117 persons stated that they did not use nets.

It is probable that any increase in the use of nets is due in large measure to the Bureau of Education. The home-economics section of this bureau requires needlework of its fifth-, sixth-, and seventh-grade girls. Since 1920 mosquito nets have been an optional part of this needlework, and since 1929 the usefulness of nets has been stressed, so that many of the girls make a net for their home as a part of their school work. Patterns are supplied them.

In our opinion it is by no means a hopeless task to persuade large numbers of people to use mosquito nets although many years and active coöperation by the Bureaus of Education and Health may be required.

At present it is difficult for the average householder to secure a suitable net. Many that are sold are of poor quality and have too large or too fine a mesh. Obviously, it is very important that proper nets be made easily available in every malarious community.

It is the purpose of this paper to suggest a suitable net made of local materials which can be sold to the people at a low price, a net which might perhaps become the standard mosquito net for rural areas in the Philippine Islands.

SINAMAY NETTING

There are many kinds of netting for sale in the Philippines, made locally, in the United States, in Europe, or in Japan. In connection with this paper we accumulated eighty-four samples by local shopping and through the courtesy of the American Trade Commissioner, the governors of several provinces, and the Bureau of Plant Industry. Many types, sizes, colors, and qualities were represented in our collection. After considerable study and having compared prices and durability, we came to the conclusion that for widespread use in the provinces there is no netting superior to sinamay netting. This material, although somewhat lacking in uniformity, has the advantages of durability, inexpensiveness, and availability. It does not soil easily and dust can be readily shaken off. It is a product of Philippines manufacture and could therefore be used widely by the Bureaus of Health and Education for standard mosquito nets without conflicting in any way with "flag" laws requiring the use of local or American products. Some of the samples from the United States were of suitable mesh and were superior to sinamay in uniformity and appearance, but were more expensive.

According to Muller(15) the word "sinamay" means a gauzy fabric and may refer to cloth made from the fibers of pineapple, banana, maguey, or abacá. Usually, however, the term "sinamay cloth" refers to that made from abacá. In our report we mean the abacá textile whenever we use the word "sinamay." Occasionally sinamay cloth contains fibers of mercerized cotton or raw silk, interwoven with those of abacá. Sinamay is sometimes woven in stripes and plaids and in some cases it is colored or has simple designs, introduced by a crude and laborious method.

The abacá of the Philippines is not hemp, although "Manila hemp" is abacá fiber. According to Miller(8) abacá is *Musa textilis*, whereas true hemp is *Cannabis sativa*. The abacá is a species of banana indigenous to the Philippines. The abacá plant resembles the banana very closely, but may be distinguished by its narrower leaves and the fact that these tend to stand more erect, instead of flopping over lazily like the banana leaf (Plate 1, fig. 1).

Abacá plants grow to a height of from 9 to 20 feet. The trunk is composed chiefly of overlapping leaf sheaths. When the flower bud appears, the entire plant is cut off close to the ground. The leaf sheaths, 5 to 12 feet long, are stripped off and separated into layers 0.25 inch or less thick, which are split into strips 1 to 2 inches wide. While still fresh and green these strips are drawn under a bolo or knife which scrapes away the pulp and leaves the fibers clean and white (Plate 2, fig. 1). As they leave the hands of the stripper the fibers are usually 9 to 12 feet long. The fiber for weaving is sold in *manojos*, or "fist bundles," usually classified into five or six grades according to diameter. Frequently, the fiber is classified and knotted at the same time by specially trained workers. The portion of the fiber from the tip of the abacá stalk is much finer than the portion from the base. The two portions are usually cut apart and similar grades tied together.

From the outside sheath to the inner ones the fiber decreases in size and strength but increases in softness. This is also true of older and younger stalks. Abacá fibers are used in rope making because of their high tensile strength, resistance to water, their length, durability, and pliability. Much of the fiber is beaten by a wooden pestle in a wooden mortar. Sometimes a grooved block is used and the fiber, placed in the grooves, is

pounded by an iron bar. The pounding tends to soften the fiber and make it more brilliant.

Looms are usually made of bamboo and may be square or cylindrical, mostly of very simple construction. The types of looms vary in different provinces. The weaving of sinamay is essentially a home industry (Plate 1, fig. 2, and Plate 2, fig. 2).

The woven cloth is usually folded and soaked in lime water for two nights. (The lime is made by burning seashells.) Then the cloth is washed in sea or other salt water and dried in the shade. Bleaching is accomplished in various ways. Fermented coconut water, a pepperlike fruit called *batuan*, and a species of citrus somewhat like an orange, are the chief bleaching agents. Cloth may be stiffened with rice starch. The final drying is done on a frame, and the cloth is then pressed under a smooth polished log.

Both Miller(8) and Minier(7) state that the weaving of abacá cloth was already a widespread industry in the Philippines when Magellan arrived in Cebu in 1521.

Our references to abacá have been the following: Muller,(15) Miller,(8) Minier,(7) Storms,(16) Buck,(17) and Mathews.(18) We have followed Miller and Minier closely in the above account.

Sinamay cloth is woven in many provinces, especially Batangas, Albay, and Sorsogon. In Alitagtag, near Taal, Batangas, for example, the residents make about 30,000 meters of all kinds of netting, chiefly abacá sinamay, per month, with about 150 individuals weaving. This amount could be doubled if the demand were present.

Sinamay is usually woven in pieces 50 cm wide. The length varies according to the demand, but is usually about 40 meters. The wholesale prices vary from 5 to 8 centavos per meter of length, occasionally as high as 11 centavos. As a rule the threads are single, but sometimes they are doubled (Plate 3 and Plate 6, fig. 1).

Sinamay cloth must be washed carefully, patted rather than rubbed. Rice starch is usually patted into the cloth after washing. In drying it must be hung carefully to put even tension on the fibers. The proper technic for washing and drying piña and sinamay is widely known in the rural Philippines.

MEASUREMENT OF MESH

There are many different kinds of mosquito netting, and a good deal of confusion has existed on the subject of measuring

this netting. MacArthur(19) was one of the first to call attention to this confusion but the situation is even worse than he reported. In that report he noted that "screencloth," or mosquito wire gauze, is measured by the number of holes per linear inch. Due regard must also be paid to the diameter of the wire. He also stated that cotton mosquito netting is not to be measured in the same way. The threads of the warp, and those of the bobbin or woof, are interwoven so that the mesh consists of two sets of parallel lines of holes intersecting one another at an angle of about 60°. These openings may be hexagonal or round. Such mesh is not measured by holes per linear inch.

In trying to determine mesh size of such cotton netting one first decides which is warp and which is woof, or bobbin. This is best done with a lens and it becomes easier with practice. Then 1 square inch of netting is marked off, and the holes along the line of the warp are counted and this count is added to the number of holes along the woof. An easy method is to cut out a hole 1 square inch in size in a piece of thin cardboard. This is then laid upon the netting so that the bottom edge is along a line of warp holes and the left hand corner at the intersection of the horizontal warp and a diagonal woof line. The mesh is the sum of the number of holes along the bottom edge of the square and the number of holes of the diagonal. The hole at the intersection is counted twice (Plate 6, fig. 2).

It is also clear that size of hole depends on size of thread. Two nets of different thread size may have the same mesh count, yet the hole of one may be small. In general, it is true that cotton netting having twenty-five to twenty-six holes, as counted above, made either of what in the trade is called "30/s" thread or of "40/60" thread, will exclude mosquitoes. But if one will consult Plates 3 to 5, inclusive, it will be seen that the matter is still more complicated. The samples of netting A to L, shown in the figures, are all made of cotton or abacá fibers and not of wire. Samples E, F, and G (Plate 4) would be counted according to the method just outlined. But how should the other samples of cloth netting be counted?

Samples A, B, and C (Plate 3) are locally made sinamay netting. In sample A the holes are rectangular with the fibers of the warp at right angles to those of the woof. The mesh of such netting would probably best be counted per linear inch along the warp and also along the woof and expressed as, for example, a mesh of 17 by 21. The same method might be ap-

plied to samples B and C, although the holes are more nearly square. Sample D is locally made cotton netting not suitable for mosquito nets. The counting of sample G and sample H, which are of Japanese weave, offer difficulties. In samples I to L, inclusive, the holes are square and the mesh of these cloth nettings should probably be counted in holes per linear inch as in wire gauze. Samples K and L are specially waxed so that they are very stiff and resemble wire netting.

It therefore appears that there are at least three ways of counting the mesh of mosquito netting, the proper method for each sample depending on the weave as follows:

1. *Holes per linear inch.*—Used for most wire netting and for cloth netting which is so woven that the threads of the warp are at right angles to those of the woof, and that the holes are square.

2. *Holes per linear inch of the warp times holes per linear inch of the woof.*—A new method suitable for Philippine sinamay netting in which the holes are rectangular, the threads of warp and woof being at right angles to each other.

3. *One line of holes along the warp added to one line of holes along the woof, within a square inch of cloth.*—Suitable for bobbinet and other cloth nettings having the threads of the woof running at an angle of about 60° to those of the warp and having roundish or hexagonal holes (Plate 6, fig. 2).

In all measurements the diameter of the fibers is important, as it obviously influences the size of the hole. It would simplify the whole matter if mosquito netting were bought and sold and experimented with on the basis of maximum and minimum diameters of the holes.

In our experiments we have followed the three methods of measuring mesh given above, but have also measured the diameters of the fibers and of the apertures.

MOSQUITO PASSAGE EXPERIMENTS

It is now a platitude in malariology that the biology of anophelines varies from country to country and even in different regions of the same country. Therefore, it is a wise procedure to make specific laboratory and field tests in dealing with malaria problems. In the present instance, for example, it seemed wise to test various nettings against the passage of local mosquitoes, although it is fairly well established in the literature that certain meshes are most suitable, on the average, to keep out mosquitoes.

Covell,(20) for instance, gives a good résumé of the references. He notes that the cloth mosquito-netting supplied to the British Army in India is 25/26 mesh made of 30/s cotton. He cites MacArthur(19) as recommending wire screening of 14 mesh, with strands of 0.0124 inch diameter, and apertures of 0.0590 inch to exclude *Aëdes*. Le Prince and Orenstein(21) found that apertures in wire screening of 0.0485 inch would permit the passage of *Aëdes* under stress of special circumstances but apertures of 0.046 inch would exclude them altogether. Earle,(22) in Puerto Rico, found that a mesh of 16, with wire diameters of 0.009 or 0.010 inch and apertures of 0.0525 to 0.0530 inch, would keep out most mosquitoes. He reported that a 14-mesh, with wire diameters of 0.015 and apertures of 0.056 inch, was sufficient for many localities.

In another communication Earle(23) reported that the least obstruction to the movement of air from a fan was caused by a 12-mesh wire screen with 0.01 inch wire. He found that in Puerto Rico a 12-mesh screen with 0.015-inch wire will keep out most insects. It is also unusually durable.

The wire screen used by the British troops in India is 14-mesh, with strand diameters of 0.014 inch, and apertures of 0.055 to 0.057 inch.

TECHNIC OF TESTS

It is true, as MacArthur(19) stated, that "we are not concerned with what mesh a frenzied mosquito imprisoned in a test tube may struggle through in order to escape from captivity, but with the mesh that a free mosquito will pass under natural conditions to obtain food." However, it is equally true that if a female mosquito of a malaria-carrying species can by its own efforts pass through a net, the mesh of that net must be considered as of doubtful safety, no matter what urge incited the insect to pull itself through the aperture. No net of any sort can be a part of "natural conditions," in the laboratory or out of it. The bed net and screened window are "unnatural" barriers between the insect and its food. It is well known that a mosquito will make persistent efforts to pass through a screen, with no greater stimulus apparently than hunger in the evening or a "homing instinct" in the early morning.

Therefore, we make no apologies for the technic of our tests, which included screened cylinders, lantern chimneys and cages, and one test of a full-size net in the field.

TABLE 1.—*Sinamay netting* [a] *(mesh 14.4 × 20.3) tested against the passage of Anopheles. Food and water attraction. Observations continued until all mosquitoes were dead.*

Experiment No.	Mosquitoes.			
	Horizontal migration.		Vertical migration.	
	Placed in chamber.	Passing the screen.	Placed in chamber.	Passing the screen.
1	45	0	53	0
2	34	0	14	0
3	23	0	35	0
4	10	0	----------	----------
Total	112	0	102	0

[a] Measurements of sinamay of Tables 1 to 6 were as follows: Average size of apertures, 0.980 by 1.280 mm; maximum size of apertures, 1.350 by 2.400; minimum size of apertures, 0.750 by 1.050; maximum diagonal, 2.8; average diagonal, 1.620.

TABLE 2.—*Species of Anopheles used in tests 1 to 4 shown in Table 1.*

Species.	Horizontal migration.		Vertical migration.		Total.
	Male.	Female.	Male.	Female.	
A. barbirostris	--------	1	--------	--------	1
A. filipinæ	6	6	3	5	20
A. ludlowi	--------	1	--------	--------	1
A. maculatus	2	8	--------	2	12
A. mangyanus	3	8	14	14	39
A. minimus var. *flavirostris*	33	26	25	35	119
A. subpictus var. *indefinitus*	5	6	--------	--------	11
Total	56	56	44	58	214

TABLE 3.—*Sinamay netting (mesh 14.4 by 20.3) tested against the passage of Culex quinquefasciatus. Food and water attraction. Observations continued until all mosquitoes were dead.*

Experiment No.	Mosquitoes.			
	Horizontal migration.		Vertical migration.	
	Placed in chamber.	Passing the screen.	Placed in chamber.	Passing the screen.
1	50	0	50	0
2	50	0	50	0
3	50	0	50	0
Total	150	0	150	0

TABLE 4.—*Sinamay netting (mesh 14.4 by 20.3) tested against the passage of Culex quinquefasciatus. Sex attraction. Horizontal migration.*

Experiment No.	Mosquitoes.					
	Placed in chambers.		Recovered dead in chambers.			
			I.		II.	
	I. Male.	II. Female.	Male.	Female.	Male.	Female.
1	39	39	39	0	0	39
2	42	42	42	0	0	42
3	40	40	40	0	0	40
Total	121	121	121	0	0	121

TABLE 5.—*Sinamay netting (mesh 14.4 by 20.3) tested against the passage of Aëdes ægypti. Food and water attraction.*

Experiment No.	Mosquitoes.					
	Horizontal migration.			Vertical migration.		
	Placed in chamber.	Passing the screen.		Placed in chamber.	Passing the screen.	
			Per cent.			*Per cent.*
1	12	1		12	1	
2	58	16		54	11	
3	52	7		32	3	
Total	122	24	19.7	98	15	15.3

TABLE 6.—*Sinamay netting (mesh 14.4 by 20.3) tested against passage of Aëdes ægypti. Sex attraction. Horizontal migration. Eight and six-tenths per cent of the males and 9.1 per cent of the females passed through the screen.*

Experiment No.	Mosquitoes.					
	Placed in chambers.		Recovered dead in chambers.			
			I.		II.	
	I. Male.	II. Female.	Male.	Female.	Male.	Female.
1	15	21	13	4	2	17
2	20	23	19	0	1	23
Total	35	44	32	4	3	40

We used two cages in our first experiments. These cages measured 18 by 18 by 36 inches. Each was divided into two equal

TABLE 7.—*Sinamay netting of various mesh sizes tested against the passage of Anopheles mosquitoes. Vertical and horizontal migration with food and water attraction.*

Test Nos.	Mesh size of netting.[a]			Species and numbers of *Anopheles* used.								
	Average.	Maximum.	Minimum.	Numbers of mosquitoes.	*A. barbirostris.*	*A. filipinæ.*	*A. ludlowi.*	*A. maculatus.*	*A. mangyanus.*	*A. minimus* var. *flavirostris.*	*A. subpictus* var. *indefinitus.*	*A. vagus* var. *limosus.*
	mm.	*mm.*	*mm.*									
1 to 5	12.7 by 14.6	13 by 15	12 by 14	Total used	16	6	------	14	16	37	------	
				Passing screen	0	1	------	0	3	11	------	0
				Passing screen—per cent	0	16.7	------	0	18.8	29.7	------	0
6 to 9	13.4 by 15.6	14 by 16	12 by 15	Total used	7	1	------	9	21	15	1	1
				Passing screen	0	0	------	0	1	2	0	0
				Passing screen—per cent	0	0	------	0	4.8	13.3	0	0
10 to 12	11.3 by 16.6	12 by 17	11 by 15	Total used	------	5	------	13	10	10	------	------
				Passing screen	------	0	------	0	0	3	------	------
				Passing screen—per cent	------	0	------	0	0	30	------	------
13 and 14	11.7 by 20.9	12 by 21	11 by 20	Total used	6	1	------	4	8	8	------	1
				Passing screen	0	0	------	0	0	0	------	0
				Passing screen—per cent	0	0	------	0	0	0	------	0
15 to 17	17.1 by 17.2	18 by 18	17 by 17	Total used	18	------	1	4	12	15	1	1
				Passing screen	0	------	0	0	0	0	0	0
				Passing screen—per cent	0	------	0	0	0	0	0	0
18 to 21	15.9 by 19.2	16 by 22	15 by 18	Total used	15	3	------	20	14	31	1	1
				Passing screen	0	0	------	0	0	0	0	0
				Passing screen—per cent	0	0	------	0	0	0	0	0

[a] Average of ten measurements at random.

compartments, one by a vertical and the other by a horizontal screen. These screens were composed of the netting to be tested. In other tests we used lantern chimneys separated by the screen to be tested. Finally we used glass cylinders, 2.75 by 3 inches, also separated by the screen to be tested. These various testing devices are shown on Plates 7 and 8.

TABLE 8.—*Sinamay netting of various mesh sizes tested against the passage of Culex mosquitoes. Vertical migration with food and water attraction. Culex quinquefasciatus.*

Test Nos.	Mesh size.[a]			Mosquitoes.		
	Average.	Maximum.	Minimum.	Used.	Passing net.	
	mm.	*mm.*	*mm.*			*Per cent.*
1 to 4	12.7 by 14.6	13 by 15	12 by 14	87	28	32.2
5 to 8	13.4 by 15.6	14 by 16	12 by 15	94	6	6.4
9 to 12	11.3 by 16.6	12 by 17	11 by 15	107	29	27.1
13 to 16	15.9 by 19.2	16 by 22	15 by 18	90	1	1.1
17 to 20	17.1 by 17.2	18 by 18	17 by 17	98	0	0.0
21 to 24	11.7 by 20.9	12 by 21	11 by 20	101	0	0.0

[a] Average of ten measurements at random.

TABLE 9.—*Sinamay netting of various mesh sizes tested against the passage of Aëdes mosquitoes. Vertical migration with food and water attraction.*

Meshes per linear inch.	Test Nos.	Mosquitoes.			
		Species.	Total.	Passing through holes of net.	
					Per cent.
Average 12.7 by 14.6	1– 4	*Aëdes albopictus*	4	4	100.0
Minimum 12 by 14		*Aëdes ægypti*	31	28	90.3
Maximum 13 by 15					
Average 11.3 by 16.6	5– 9	*Aëdes albopictus*	12	5	41.7
Minimum 11 by 15		*Aëdes ægypti*	32	15	46.9
Maximum {12 by 17 / 11 by 18}					
Average 13.4 by 15.6	10–14	*Aëdes albopictus*	8	3	37.5
Minimum 12 by 15		*Aëdes ægypti*	28	16	57.1
Maximum 14 by 16					
Average 15.9 by 19.2	15–19	*Aëdes albopictus*	21	7	33.3
Minimum 15 by 18		*Aëdes ægypti*	42	9	21.4
Maximum 16 by 22					
Average 11.7 by 20.9	20–24	*Aëdes albopictus*	7	0	00.0
Minimum {11 by 21 / 12 by 20}		*Aëdes ægypti*	52	9	17.3
Maximum 12 by 21					
Average 17.1 by 17.2	25–29	*Aëdes albopictus*	5	0	00.0
Minimum 17 by 17		*Aëdes ægypti*	35	3	8.6
Maximum 18 by 18					

In our first test we used a local sinamay cloth having an average mesh of 14.4 by 20.3. This netting is being used for mosquito nets in some localities. The results of tests with *Anopheles* mosquitoes are shown in Tables 1 and 2; with *Culex* mosquitoes in Tables 3 and 4; and with *Aëdes* mosquitoes in Tables 5 and 6. In the case of *Anopheles* the tests were on the basis only of food and water attraction, the mosquitoes being placed in one compartment and a cut mango and water in the other compartment. In these tests no *Anopheles* made either vertical or horizontal passage through the netting.

Culex and *Aëdes* were tested in the same way for vertical and horizontal passage. They were also tested by sex attraction, males being placed in one chamber and females in the other. In no test did any *Culex quinquefasciatus* pass through the netting, but some *Aëdes ægypti* succeeded in passing this mesh in each test. All of the mosquitoes used in these tests were bred out in the laboratory.

Measurements of sinamay of Tables 7, 8, and 9 were as follows:

Average mesh per linear inch.	Average size of apertures in millimeters.
12.7 by 14.6	1.435 by 1.745
11.3 by 16.6	1.240 by 1.540
13.4 by 15.6	1.340 by 1.615
15.9 by 19.2	1.180 by 1.260
11.7 by 20.9	1.110 by 1.660
17.1 by 17.2	1.140 by 1.360

TABLE 10.—*Measurements of dead mosquitoes (greatest diameter in millimeters).*[a]

Species.	Maximum.	Minimum.	Average.
Lutzia fuscana	3.40	2.22	2.31
Culex quinquefasciatus	1.59	1.52	1.56
Anopheles barbirostris	1.46	1.17	1.33
Anopheles filipinæ	1.11	0.86	0.96
Anopheles maculatus	1.18	1.04	1.11
Anopheles mangyanus	1.20	0.94	1.09
Anopheles minimus var. *flavirostris*	1.10	0.85	0.99
Aëdes ægypti	1.37	1.01	1.14
Aëdes albopictus	1.50	1.11	1.29

[a] These measurements were made on dried specimens several days old. Ten female mosquitoes of each species were measured, several measurements of each specimen being taken. Bred out mosquitoes were used. In these specimens the greatest diameter was always through the thorax and included the coxa and a portion of the trochanter of the middle leg on the side measured. See diagram. (Courtesy of Mr. J. Espinosa, division of weights and standards, Bureau of Science.)

TABLE 11.—*Measurements of living female mosquitoes.*[a]

Species.	Source.	Number.	Measurement of thorax.		
			Maximum.	Minimum.	Average.
			mm.	*mm.*	*mm.*
Anopheles filipinæ	Caught wild	10	1.20	1.10	1.14
Anopheles mangyanus	do	18	1.30	1.12	1.19
Anopheles minimus var. *flavirostris*	do	81	1.25	1.03	1.13
Do	Bred out	6	1.10	0.95	1.06
Culex quinquefasciatus	do	100	1.85	1.45	1.72
Aëdes ægypti	do	35	1.73	1.45	1.58

[a] The anophelines were from Laguna and Nueva Vizcaya Provinces. The culicines were from Pasay, Rizal, and the aëdines from Manila. The greatest diameter was taken. This was always through the thorax and included the coxa and a portion of the trochanter of the middle leg on the side measured. Living mosquitoes quieted by chloroform, were used. (Courtesy of Mr. F. E. Baisas, Malaria Investigations laboratory.)

Tables 7, 8, and 9 summarize the results of numerous tests with *Anopheles, Culex,* and *Aëdes,* respectively, against various sizes of sinamay netting. From Table 7 it will be seen that anophelines do not pass netting having a mesh of 11.7 by 20.9 or more. But malaria-carrying *A. minimus* var. *flavirostris* will pass a mesh of 11.3 by 16.6. Of the three mosquitoes of this species passing this netting, two were females and one was a male.

From Table 8 it will be seen that *Culex quinquefasciatus* did not succeed in passing the sinamay netting having an average of 17.1 by 17.2 or 11.7 by 20.9. One of 90 passed through a net of 15.9 by 19.2 average mesh.

From Table 9 it will be seen that aëdines were able to pass apertures which culicines and anophelines could not pass.

In Tables 10 and 11 are given the results of some measurements of living and dead female mosquitoes of several species (text fig. 1). It will be seen from these tables that, unfortunately, the malaria-carrying species of the Philippines are among the smallest mosquitoes. In Table 10, for example, dead females of *A. minimus* var. *flavirostris,* the chief carrier, had an average largest diameter of 0.99 mm. The living specimens of this species, as shown in Table 11, had an average diameter of 1.13, wild-caught, and 1.06, bred out. The apertures of our proposed standard net average 1.18 by 1.215 mm, so that, theoretically, *A. minimus* var. *flavirostris* could pass the apertures. Actually, in all of our laboratory tests and our field trials, no

passing occurred; but these measurements indicate that the mesh chosen is at about the theoretical limit desirable. Comfort requirements make advisable the largest safe mesh.

It is apparent that some species of mosquitoes are more agile than others in penetrating a net. Just as *Aëdes ægypti* has become more adapted to house conditions, is more clever about attacking from the rear, and is more elusive to hand-catching, so it is cleverer in getting through a net. With an average greatest diameter of 1.58 mm it will pass apertures averaging 1.18 by 1.215 mm. We have made special observations to see why this should be so, and we find that *Aëdes ægypti* utilizes the diagonal size of the aperture. A rectangular hole measuring 1.18 by 1.215 mm has a diagonal measurement of 1.694 mm, and this is sufficient for *Aëdes* averaging 1.58 mm. Several times we have observed *Aëdes* passing such a net. The insect at first apparently searches for the most suitable aperture. It then pushes one foreleg and its head through this hole. Then it hooks the tibia of its protruding foreleg over a thread, and pulls itself through, making full use of the diagonal. Rarely, both forelegs protrude with the head and are hooked over a thread. The mid and hind legs and the wings offer no difficulties folded back along the abdomen. The insect, however, is not infallible. Sometimes it inserts the forelegs in holes adjacent to the one it is trying to pass. This naturally complicates matters so that there is failure to pass.

In one case we measured an aëdine that we had seen pass a certain hole, which we also measured. The greatest diameter of the mosquito was 1.73 mm. The rectangular hole measured 1.70 by 1.65 mm with a diagonal of 2.36 mm. The mosquito came through without any difficulty, its sternum turned toward one corner and its dorsum toward the opposite corner.

FIELD TEST

A net made of sinamay having a mesh of 15.9 by 19.2, threads averaging 0.328 mm, and apertures averaging 1.18 by 1.25 mm, was tested in Calauan, Laguna, where *Culex, Aëdes,* and the *funestus-minimus* subgroup of anophelines are abundant. It was used for ten nights with careful observations during each night and morning. No mosquitoes entered the net during this period to feed on the person sleeping within it. Daily collections of *funestus-minimus* adults were made in natural daytime shelters nearby during the period of observation.

TABLE 12.—*Passage of wind through netting using fan alone. No tunnels to straighten wind current.*

Test No.	Conditions.	Anemometer readings. M/min.[a]						Effect of screen.	
		1	2	3	4	5	Average.	Difference.	Wind passing screen.
								M/min.	*Per cent.*
1	No screen	102	117	118	90	91	104		
	18-mesh copper	0	0	0	0	0	0	104	00.0
2	No screen	90	120	130	130	121	118		
	No. 2 sinamay	0	0	0	0	0	0	118	00.0
3	No screen	102	118	120	117	95	110		
	No. 6 cotton	0	0	0	0	0	0	110	00.0
4	No screen	220	218	216	220	218	218		
	18-mesh copper	75	74	75	78	80	76	142	34.9
5	No screen	210	212	220	208	212	212		
	No. 2 sinamay	41	40	45	40	42	42	170	19.8
6	No screen	215	220	218	216	200	214		
	No. 6 cotton	27	25	20	28	30	26	188	12.1

[a] Meters per minute.

TABLE 13.—*Passage of wind through netting. Wind from fan straightened by tunnels.*[a]

Test No.	Conditions.	Anemometer readings. M/min.						Effect of screen.	
		1	2	3	4	5	Average.	Difference.	Wind passing screen.
								M/min.	*Per cent.*
1	No screen	98	90	92	97	102	96		
	18-mesh copper	70	73	75	79	78	75	21	78.1
2	No screen	105	107	110	106	104	106		
	No. 2 sinamay	78	79	79	83	77	79	27	74.5
3	No screen	109	112	113	115	114	113		
	No. 6 cotton	70	74	72	73	73	72	41	63.7
4	No screen	177	175	178	182	180	178		
	18-mesh copper	124	130	131	128	128	128	60	71.9
5	No screen	177	188	186	185	197	187		
	No. 2 sinamay	127	127	120	125	127	125	62	68.9
6	No screen	185	187	187	192	195	189		
	No. 6 cotton	118	117	117	124	125	120	69	63.5

[a] The No. 2 sinamay mesh was on the average 15.9 by 19.2 with a minimum of 15 by 18 and a maximum of 16 by 22. The No. 6 cotton cloth had a mesh of 17 by 21 with rectangular holes. The strand sizes were: Copper wire, 0.298 mm; sinamay fibers, 0.328 mm; cotton fibers, 0.172 mm.

WIND PASSAGE EXPERIMENTS

In Tables 12 and 13 are given the results of some tests of various kinds of netting as regards the amount of wind that they would allow to pass. An electric fan was used as the source of wind, and this appears to have been the usual proce-

dure in similar experiments reported elsewhere. Table 10 gives the results when the fan was used, blowing directly on the net at right angles to the blades of the fan. Tests were made with three materials as follows:

A. 18-mesh copper.
Average diameter of wire, 0.298 mm (0.0117 inch).
Average diameter of aperture, 1.068 by 1.100 mm (0.0421 by 0.0433 inch).

B. Sinamay netting No. 2. Mesh, 15.9 by 19.2.
Average diameter of fibers, 0.328 mm (0.0129 inch).
Average diameter of aperture, 1.180 by 1.215 mm (0.0465 by 0.0479 inch). (Rectangular.)

C. Cotton netting No. 6. Mesh, 17 by 21.
Average diameter of fibers, 0.212 mm (0.0084 inch).
Average diameter of aperture, 1.307 by 0.960 mm (0.0515 by 0.0388 inch). The mesh of this cotton netting was measured like that of sinamay because the warp and the woof were at right angles to each other and the holes rectangular.

In each case tests were made at two speeds of the fan. In each test the speed of the wind from the fan was first measured in meters per minute, five times without the screen and then immediately five times with the screen interposed between fan and anemometer, an average speed being determined in this way in each test.

It will be seen in Table 12 that when the wind velocity was 104, 118, and 110 meters per minute without the screen, it became zero with a screen, whether the screen was of copper, sinamay, or cotton. In other words 100 per cent of the wind was kept out by the screen. At velocities of 218, 212, and 214 meters per minute some of the wind passed the screen, as follows:

Material.	Wind passing through. Per cent.
Wire screen	34.9
Sinamay netting	19.8
Cotton netting	12.1

Wind as it leaves a fan must be swirling and is probably not traveling in a straight line, as does wind coming into a room where a bed net is in use. Therefore, a second series of tests were made with a number of paper tunnels placed between the fan and the screen. These tunnels were 11 inches long and in tests 1 to 3 had diameters averaging 0.8 inch. In tests 4 to 6 the tunnels were of the same length, but had diameters averag-

ing 1.28 inches. The purpose of these tunnels was to straighten out the wind currents so that they would strike the netting at right angles to the apertures, as would normally be the case with a net hanging in a room exposed to a natural wind. Table 13 gives the results of these tests, and it will be seen that there was a marked difference. In tests 1 to 3, Table 13, the average velocity of the wind without a screen was 96, 106, and 113 meters per minute. Although in the first series (Table 12), the screens allowed no wind to pass at approximately these velocities, we found that having straightened the currents with the tunnels, a considerable amount of wind passed through the screens, as follows:

Material.	Wind passing through. Per cent.
Copper wire	78.1
Sinamay	74.5
Cotton	63.7

With the larger tunnels (Table 11, Nos. 4–6) the velocities without the screen were 178, 187, and 189. About the same percentage of wind passed the screens, as follows:

Material.	Wind passing through. Per cent.
Copper wire	71.9
Sinamay	68.9
Cotton	63.5

Therefore, it would appear that, in testing netting for wind passage, if an electric fan is employed the wind currents must be straightened by some such device as we have used. Simple confirmation of the fact that much of the air directly from the fan is deflected when it strikes the net, may be had by placing the hand to one side of the screen being tested. Without the tunnels a strong current of deflected air is felt. With the tunnels the volume of deflected air is noticeably very much less. The swirling of the air currents from a fan may also be demonstrated by blowing smoke in front of the blades of the fan. We are indebted to Capt. Guy Hill, United States Army, for suggestions in regard to the use of tunnels in these tests.

These tests show that sinamay netting is better than cotton netting as regards the passage of wind. Wire netting is better than sinamay. Sinamay appears to keep out about 25 to 30 per cent of the wind that strikes the net squarely.

TABLE 14.—*Effect of screening on wet-bulb thermometer readings. Wind direct from fan. No tunnels.*

Conditions.	Time required to lower wet bulb to minimum.[a]	
	First series.	Second series.
	Seconds.	*Seconds.*
No screen	50	55
Do.	50	55
18-mesh copper	95	100
No. 2 sinamay	106	105
No. 6 cotton	120	125

[a] During these tests the maximum dry-bulb reading was 83.8; minimum, 83. The maximum wet-bulb reading was 78.8; minimum, 77. The actual sizes of the screens used are given in Table 18.

TABLE 15.—*Effect of screening on wet-bulb thermometer readings. Wind from fan straigtened by tunnel.*

Conditions.	Time required to lower wet bulb to minimum.[a]	
	First series.	Second series.
	Seconds.	*Seconds.*
No screen	45	50
18-mesh copper	55	50
No. 2 sinamay	60	55
No. 6 cotton	75	70

[a] During these tests the maximum dry-bulb reading was 84.2; minimum, 84. The maximum wet-bulb reading was 79, minimum, 77.9. The actual sizes of the screens used are given in Table 18.

WET- AND DRY-BULB EXPERIMENTS

In Tables 14 and 15 are given the results of some tests with a wet-bulb thermometer, and in Tables 16 and 17 tests with the wet- and dry-bulb Kata-thermometer of Leonard Hill. These readings might be called comfort or evaporation tests. The tests were made with fans with and without the tunnels mentioned above. Here again it will be seen that the effect of the tunnels was marked, more wind passing through the various nets when the tunnels were used to straighten out the air currents. In all of the tests the degree of comfort indicated was greatest in the wire netting and least in the cotton netting. In Tables 14 and 15 are given the number of seconds required to lower the wet-bulb thermometer to its minimum reading. Using the

tunnels, it required between 45 and 50 seconds to lower the thermometer reading to its minimum without a screen, 50 to 55 seconds with a wire screen, 55 to 60 with sinamay, and 70 to 75 with cotton netting. This would seem to indicate that a sinamay netting might reduce the comfort of a sleeping person by about 14 per cent. Considering the fact that the average rural dweller in the Philippines in the past has as a rule closed all windows at night, it is not likely that this difference would be very noticeable. Certainly, freedom from the bites of mosquitoes and obviating the need for covering the face with a blanket, as has been a common habit, would more than compensate for this difference in comfort between no net and a sinamay net. Wire screening would no doubt be more comfortable than a sinamay bed net, but, as explained above, the average rural house in the Philippines could not be screened at a reasonable cost.

TABLE 16.—*Tests of netting with Kata-thermometer. Without tunnels.*

Description.[a]	Without screen.	18-mesh wire.	No. 2 sinamay.	No. 6 cotton.
Dry Kata-thermometer:				
Time in seconds	127.5	151.5	161.0	176.5
Cooling power in millicalories	3.7	3.1	2.9	2.6
Wet Kata-thermometer:				
Time in seconds	27.0	30.0	32.0	38.5
Cooling power in millicalories	17.3	15.5	14.5	12.1

[a] The time in seconds is the time required to reduce the thermometer fluid from 100° to 95° C. The cooling power of the dry Kata is that by radiation and convection. The cooling power of the wet Kata is that by radiation, convection, and evaporation. The figures in millicalories are computed from the table supplied with the instrument in millicalories per square centimeter per second. The size of the mesh is given in Table 18. Room temperature, dry bulb, 29.5° C.; wet bulb, 26° C.

TABLE 17.—*Tests of netting with Kata-thermometer, using tunnels to straighten wind currents.*

Description.[a]	Without screen.	18-mesh wire.	No. 2 sinamay.	No. 6 cotton.
Dry Kata-thermometer:				
Time in seconds	86.5	106.0	126.5	131.5
Cooling power in millicalories	5.4	4.4	3.7	3.5
Wet Kata-thermometer:				
Time in seconds	20.5	25.5	28.0	29.5
Cooling power in millicalories	22.7	18.3	17.0	16.0

[a] The note of Table 16 applies to this table also. We are indebted to Dr. S. Arima for his assistance in the tests summarized in Tables 16 and 17.

TABLE 18.—*Measurements of screening.*[a]

No.	Copper, 18-mesh.			Sinamay, No. 2.			Cotton, No. 6.			
	Wire.	Aperture.		Thread.	Aperture.		Thread.		Aperture.	
		1	2		1	2	1	2	1	2
1	18	65	68	19	73	60	15	13	71	60
2	19	62	66	15	60	84	17	17	76	59
3	19	63	63	28	89	56	11	19	81	60
4	18	67	65	19	65	90	8	11	77	58
5	17	58	68	16	65	70	10	15	75	60
6	17	60	66	24	75	84	8	14	85	51
7	16	67	62	30	66	83	6	18	65	50
8	19	67	67	19	67	70	9	12	77	60
9	17	67	67	30	66	52	8	18	87	53
10	19	65	68	16	82	80	11	14	80	65
11	------	------	------	18	------	------	------	------	------	------
12	------	------	------	22	------	------	------	------	------	------
13	------	------	------	13	------	------	------	------	------	------
14	------	------	------	17	------	------	------	------	------	------
15	------	------	------	15	------	------	------	------	------	------
16	------	------	------	21	------	------	------	------	------	------
17	------	------	------	16	------	------	------	------	------	------
18	------	------	------	17	------	------	------	------	------	------
19	------	------	------	25	------	------	------	------	------	------
20	------	------	------	14	------	------	------	------	------	------
Total	179	641	660	394	708	729	103	151	784	576
Average	17.9	64.1	66.0	19.7	70.8	72.9	10.3	15.1	78.4	57.6
Average, mm	0.298	1.068	1.100	0.328	1.180	1.215	0.172	0.252	1.307	0.960

[a] Except in the bottom row all figures are in micrometer spaces. One space equals 1/60 millimeter. The bottom row gives average figures in millimeters. The No. 1 columns refer to warp threads or to apertures along the line of the warp. The No. 2 columns refer to the woof. The mesh of sinamay sample No. 2 was 15.9 by 19.2 and that of cotton Number 6 was 17 by 21, the holes in each case being rectangular. (For conversion of millimeters to inches multiply by 0.0394.)

In Tables 16 and 17 are the results of the Kata wet-bulb and dry-bulb thermometer readings. The dry-bulb readings are a measure of heat loss by radiation and convection. The wet-bulb readings not only give a measure of loss by radiation and convection but also by evaporation. As in the other tests, it will be noted that the best netting, as regards comfort, is the wire screen. The sinamay is second, and the cotton netting third.

In these tables the heat loss in millicalories per square centimeter per second is estimated, using the factor 466 assigned to the particular Kata-thermometer we used. This factor divided by the cooling time in seconds gives the estimated heat loss. It will be seen that in these tests the screens slowed

down the heat loss, cotton netting by as much as 30 to 35 per cent and wire netting by as little as 10 to 20 per cent, approximately. The sinamay netting slowed the time by from 15 to 25 per cent, approximately.

Many more readings would have to be taken before the loss of comfort due to netting of a particular make could be stated definitely, but the tests reported are sufficient to indicate clearly the relative status of wire, sinamay, and cotton, and to demonstrate that the comfort loss although real is not great enough to be a serious drawback to the use of a sinamay net.

A STANDARD MOSQUITO BED NET

There are certain points about a mosquito net for use with beds, as noted by various observers and summarized by one of us,(24) that require emphasis.

(*a*) The size of mesh is important. The optimum is the size that is just small enough to exclude mosquitoes. A smaller mesh tends to curtail the circulation of air unnecessarily and will not be used consistently by laymen.

In the Philippines we are interested principally in excluding anophelines of the *funestus-minimus* subgroup and *Anopheles maculatus*. From our experiments we believe that sinamay netting of about 16 by 20 average mesh, with fibers averaging 0.328 mm, and with average apertures of 1.180 by 1.215 mm, will exclude all anophelines, all culicines, and most aëdines. We believe such sinamay netting to be a satisfactory material for local mosquito nets.

(*b*) It is best to use white netting, as mosquitoes are more easily seen against such a background. Not only the sides but also the top of the mosquito bar should be of netting, for the sake of better circulation of air. When cotton is used the bottom should be of strong material, and the seams require reënforcement with tape. Sinamay nets do not require tape at the seams.

Sinamay netting is not white but a light cream color. It is occasionally dyed in various colors, but the undyed netting is best. While it might be somewhat better if the bottom of the net were made of some such material as calico, it is not necessary and it increases the cost of the net. The entire net may well be made of sinamay.

(*c*) The shape of the net requires attention. It should not be conical but rectangular. No openings should be provided for entrance or exit. To enter a net the user should lift the bottom

carefully and should slip inside quickly. Of course, no rent or wide separation of fibers should be tolerated, as mosquitoes will spend much time searching for such places.

(*d*) The hanging of the net is also important. Sometimes a wide four-poster bed can be used, the net being hung inside the posts and carefully tucked under the mattress all around. Nets should not sag or hang loosely. They should be put in place before dark and always searched for stray mosquitoes. The top should be about 4 feet above the bed, and there should be a foot of excess cloth at the bottom to be tucked under the mattress.

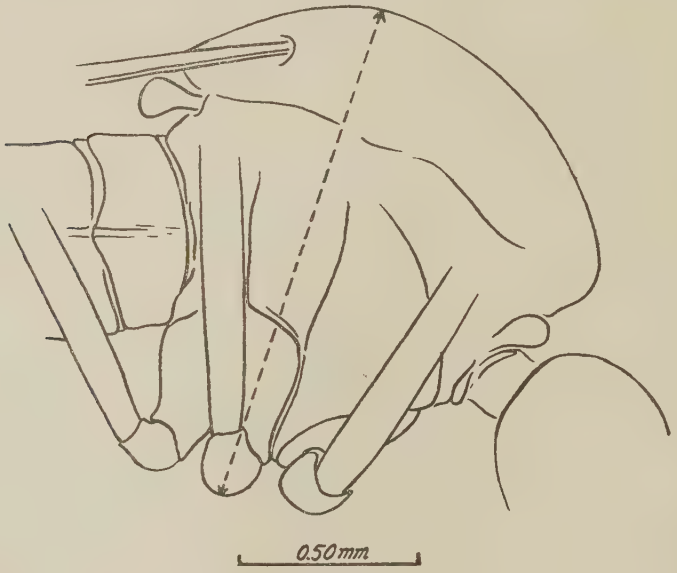

FIG. 1. Diagrammatic sketch showing line along which greatest diameter of mosquitoes was measured.

When the net is to be hung outside the bed posts it must be much longer, for at least a foot of excess must rest on the floor. In this case there is great danger of mosquitoes hiding under the bed and being inclosed with the sleeper in the net. Only very careful spraying and brushing will drive out these net mates, and it is for this reason that tuck-in nets are usually more effective. There is better circulation of air under the larger nets, however.

In the Tropics most persons, especially in the country, sleep without mattresses, so that there is nothing under which to tuck a mosquito net. Moreover, floors may be loosely made, so that a net hanging outside the bed to the floor is poor protection.

The beds also are often very loosely made. These conditions obtain in the rural Philippines. In such cases it is well to advise that a closely woven mat (*banig*) cover the surface of the bed or floor. Then a mosquito net may be used which has tunnels at the level of the bed parallel to the four lower edges of the net. Through these tunnels bamboo poles may be placed (Plates 9 and 10) or the tunnels may be weighted with stones. At least a foot of excess netting must be available below the tunnel to be tucked carefully under the bamboo poles or stones. A wide, long bed is advisable, so that elbows or knees or toes will not press against the net. Such a country net has been successfully used in the Dutch East Indies and would do very well in rural Philippines. They are especially suitable for those who sleep on the floor. We do not know to whom belongs the credit for this idea. In 1927, one of us (P. F. R.) saw such nets in use in Java.

It is a health officer's duty to make practical mosquito nets available to his people. Arrangements for the manufacture and the sale of nets can always be made without much difficulty and at reasonable cost. It seems to us that the first step in malaria prophylaxis to be undertaken by a health officer in the Philippines should be the making of necessary arrangements for the sale of suitable mosquito nets locally. Then this officer should actively and energetically attempt to persuade his constituents to buy and to use them.

SIZE OF STANDARD NET

We propose for a standard net for the use of one person the following dimensions: Length, 188 cm; width, 100; height, 150; tunnel width, 12. This would require 23 meters of sinamay netting, 50 cm wide, as usual. Each side would consist of three strips of sinamay, each 50 cm wide, sewn together lengthwise to make the height of 150 cm, requiring 3 by 188, or 564 cm, for each side, a total of 1,128 cm for both sides. Each end would consist of three strips of sinamay (continuations of the side) each 50 cm wide; that is, 3 by 100 or 300 cm for each end, a total of 600 cm for the ends. The top would require two strips, each 188 cm long, a total of 376 cm. The tunnel would require one strip 50 cm wide and 188 cm long cut lengthwise into four pieces each 12 cm wide, two being 188 cm long and two being 100 cm long. This would leave a piece of netting 88 by 25 cm

for loops. To summarize, the amount of netting, 50 cm wide, needed would be for the sides, 1,128 cm; ends, 600; top, 376; tunnel, 188; total, 2,292 cm.

Therefore, 23 meters of sinamay netting would make a net suitable for one person sleeping on either a single or a double native bed. These beds generally measure about as follows: Single native bed (*papag*), 165 cm long, 67 cm wide, 50 cm high. Double native bed, 185 cm long, 110 cm wide, 50 cm high.

According to studies by Nañagas and Santiago(25) the average height of male Filipinos is about 164 cm, and of females about 153 cm. Therefore, a net of 188 cm allows leeway for putting the arms above the head.

The 23 meters of sinamay netting would cost from 1.15 to 1.84 pesos,[1] as the price varies from 5 to 8 centavos per meter. It should be possible in most places to get the netting for 6 centavos per meter. It costs about 20 to 25 centavos to sew one net. Therefore, the total cost of a net would be from 1.35 to 2.09 pesos, depending on the cost of material and sewing. It is probably safe to say that the average cost of such a standard net as we recommend would be about 1.50 pesos.

Increasing the width of the net to 150 cm would make the dimensions suitable for two persons. Such a double net would require 28 meters of material, increasing the price of the net from 25 to 40 centavos. For persons sleeping on the floor the height could be decreased to 100 cm. When several persons sleep together on the floor, as is not uncommon in rural houses, a room net may be used. One measuring 350 by 250 by 100 cm can be made for 3.50 pesos.

The mesh of the sinamay should not count less than 16 or more than 20 in either direction per linear inch or 2.5 cm. The apertures should not be less than 1.15 mm or more than 1.5 mm in either dimension. The fiber should average about 0.328 mm.

SUMMARY

This paper reviews the subject of mosquito nets, describes local sinamay netting made from abacá fiber, describes some experiments with various nettings as regards mosquito passage, wind passage, and comfort, and presents for the rural Philippines a possible standard mosquito bed net that would be

[1] One peso Philippine currency equals 100 centavos or 50 cents United States currency.

cheap, durable, made of local materials, and effective in malaria prophylaxis.

CONCLUSIONS

1. Mosquito nets have certainly been used in the Philippines since 1640 and were probably used to some extent even prior to the Spanish era, which began in 1521.

2. The type of rural house in the Philippines is such that screening is not feasible. There is reason to believe that bed nets offer the most practical means at the disposal of the average householder for malaria prophylaxis.

3. The mesh of various kinds of netting must be measured in one of at least three different ways, depending on (*a*) whether the warp is or is not at right angles to the woof and (*b*) whether the holes are square or (*c*) rectangular. It would simplify matters considerably if mosquito netting were bought and experimented with on the basis of size of aperture.

4. The largest diameters of living, caught-wild anophelines of the *funestus-minimus* subgroup average as follows: *Anopheles filipinæ*, 1.14 mm; *A. mangyanus*, 1.19; *A. minimus* var. *flavirostris*, 1.13. Certain other species average as follows: *Anopheles barbirostris* (dead), 1.33 mm; *A. maculatus* (dead), 1.11; *Culex quinquefasciatus* (living), 1.72; *Aëdes ægypti* (living), 1.57.

5. Aëdes mosquitoes in passing through a net utilize the diagonal of an aperture. Having pushed head and one foreleg into the hole they hook the tibia of the protruding leg over a thread and pull the rest of their body through.

6. Locally made sinamay netting having a mesh of about 16 by 20, with fibers averaging 0.328 mm and apertures averaging 1.180 by 1.215 mm, will keep out all anophelines, all culicines, and most aëdines. It is a cheap, durable, and practical material for local mosquito nets. It will not admit as much wind as copper-wire screening of about the same size but is better than cotton netting in this respect.

7. Wind currents from an electric fan do not travel in a direct line but are swirling. To test the qualities of a screen as regards wind passage it is essential to straighten the wind currents, by using some such device as tunnels, in order to approximate the conditions prevailing when natural wind strikes a bed net.

8. As regards wind passage and comfort, wire screening is better than sinamay and sinamay is better than cotton netting.

9. Sinamay netting probably reduces comfort by not more than 25 per cent (in some tests as little as 14 per cent).

10. A standard mosquito net for the Philippines might have the following dimensions: Single, length, 188 cm; width, 100; height, 150. Double, length, 188 cm; width, 150; height, 150.

Such nets made of sinamay would cost about 1.50 and 1.75 pesos, respectively. They should be specially constructed for use where beds have no mattresses and where the people have no beds. The mesh should not be less than 16 or more than 20 apertures in either direction per linear inch or 2.5 cm, with apertures not less than 1.15 mm or greater than 1.5 mm in either direction. Fibers should average about 0.328 mm.

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ILLUSTRATIONS

PLATE 1

FIG. 1. Abacá plants (*Musa textilis*). (Courtesy of the Bureau of Science.)
2. Weaving on a hand loom, Philippine Islands. (Courtesy of Dr. F. Vanoverbergh.)

PLATE 2

FIG. 1. A woman stripping abacá fibers. (Courtesy of the Bureau of Science.)
2. Weaving abacá sinamay on a hand loom, Alitagtag, Batangas.

PLATE 3

Philippine hand-woven netting; *a*, *b*, and *c* are all abacá sinamay; *d* is cotton. (Inch ruler.)

PLATE 4

Imported netting; *e* and *f* are English manufacture; *g* and *h* are Japanese manufacture; *e* has the usual 25/26 mesh for malaria prophylaxis. (Inch-ruler.)

PLATE 5

FIGS. *i* and *j*. Imported netting of Japanese manufacture. (Inch ruler.)
k and *l*. Imported netting of United States manufacture. Waxed cloth resembling wire.

PLATE 6

FIG. 1. Sinamay netting showing detail. In this sample the woof threads are double.
2. Diagram showing method of counting mesh when the woof is at an angle of 60° with the warp. Count holes *a* to *b* and add to holes *b* to *c*. Hole at *b* is to be counted twice. One square inch is shown. This sample counts 23.

PLATE 7

Cages for testing migration of mosquitoes through netting. Top cage for vertical migration, bottom cage for horizontal. Each cage has two compartments separated by the screen being tested. Only the first compartment in the lower cage is clearly shown in the photograph.

PLATE 8

Chimneys and cylinders used for testing migration of mosquitoes through netting.

PLATE 9

FIG. 1. Sinamay mosquito net inclosing single rural-style bed. The tunnels at bottom contain bamboo poles. The bottom of the net is tucked under this weight.

2. Sinamay mosquito net used on a double rural-style bed. The net is held in place by bamboo poles in tunnels as explained in the text.

PLATE 10

Detail of corner of bed and mosquito net, showing position of bamboo poles in the net tunnels under which the bottom of the net is tucked.

TEXT FIGURE

FIG. 1. Diagrammatic sketch showing line along which greatest diameter of mosquitoes was measured.

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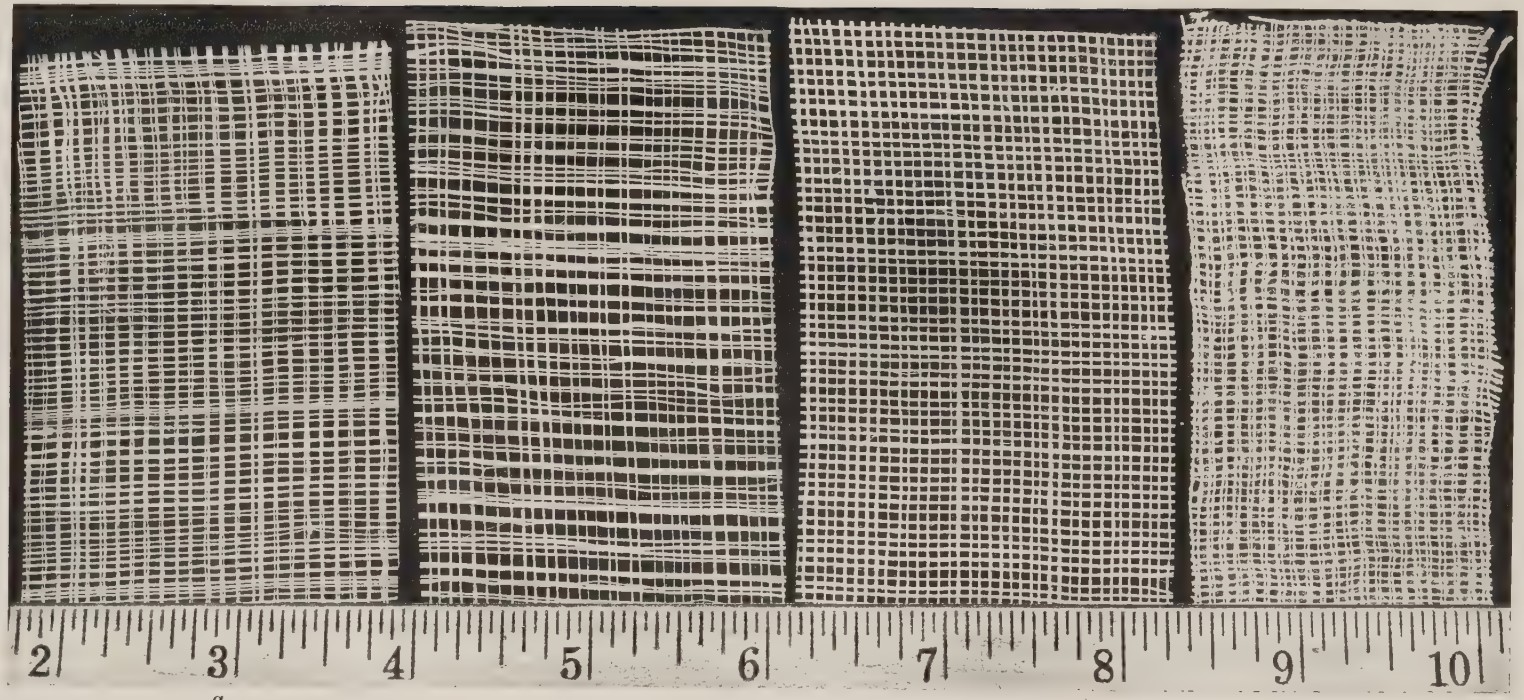
PLATE 1.

1

2

PLATE 2.



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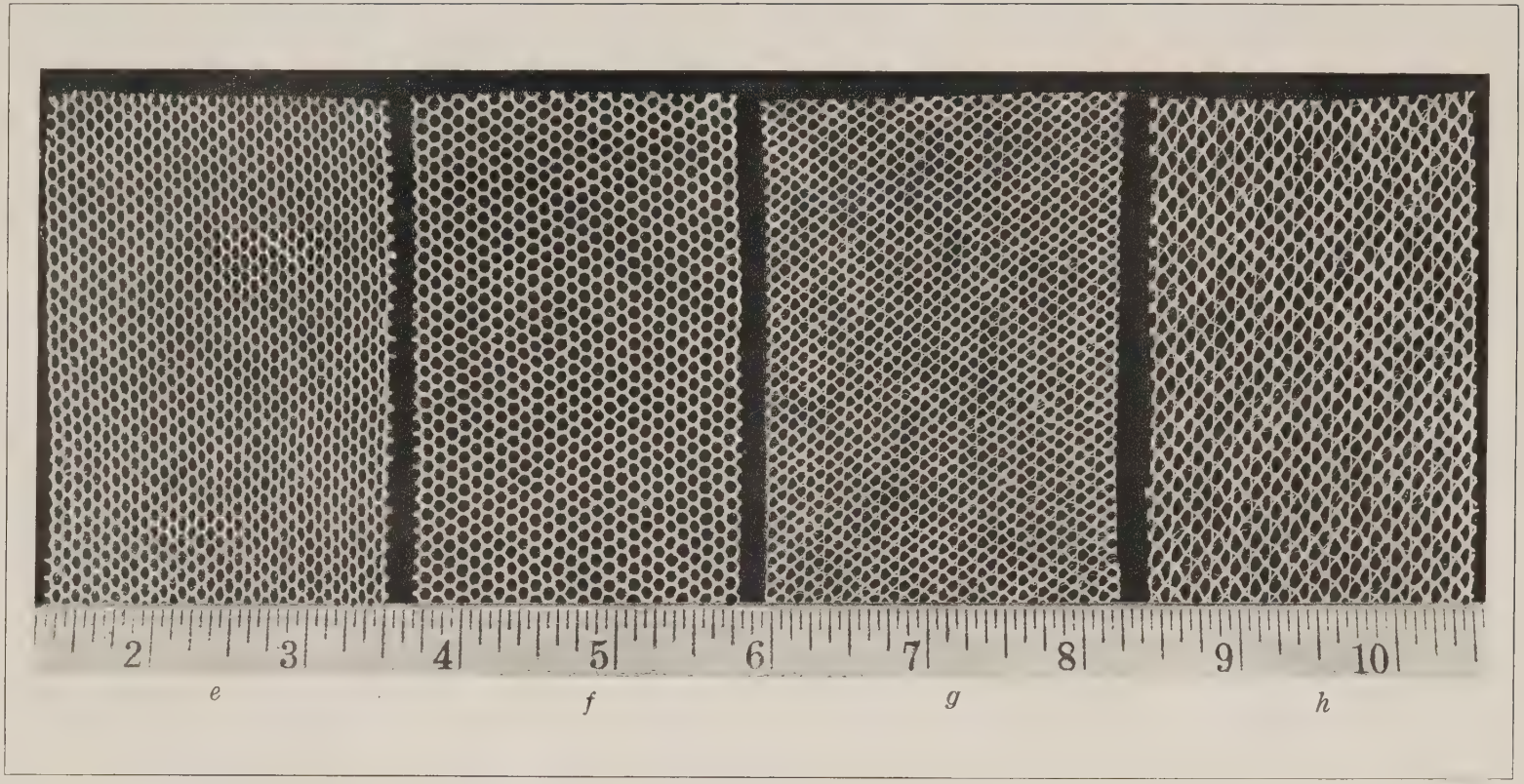

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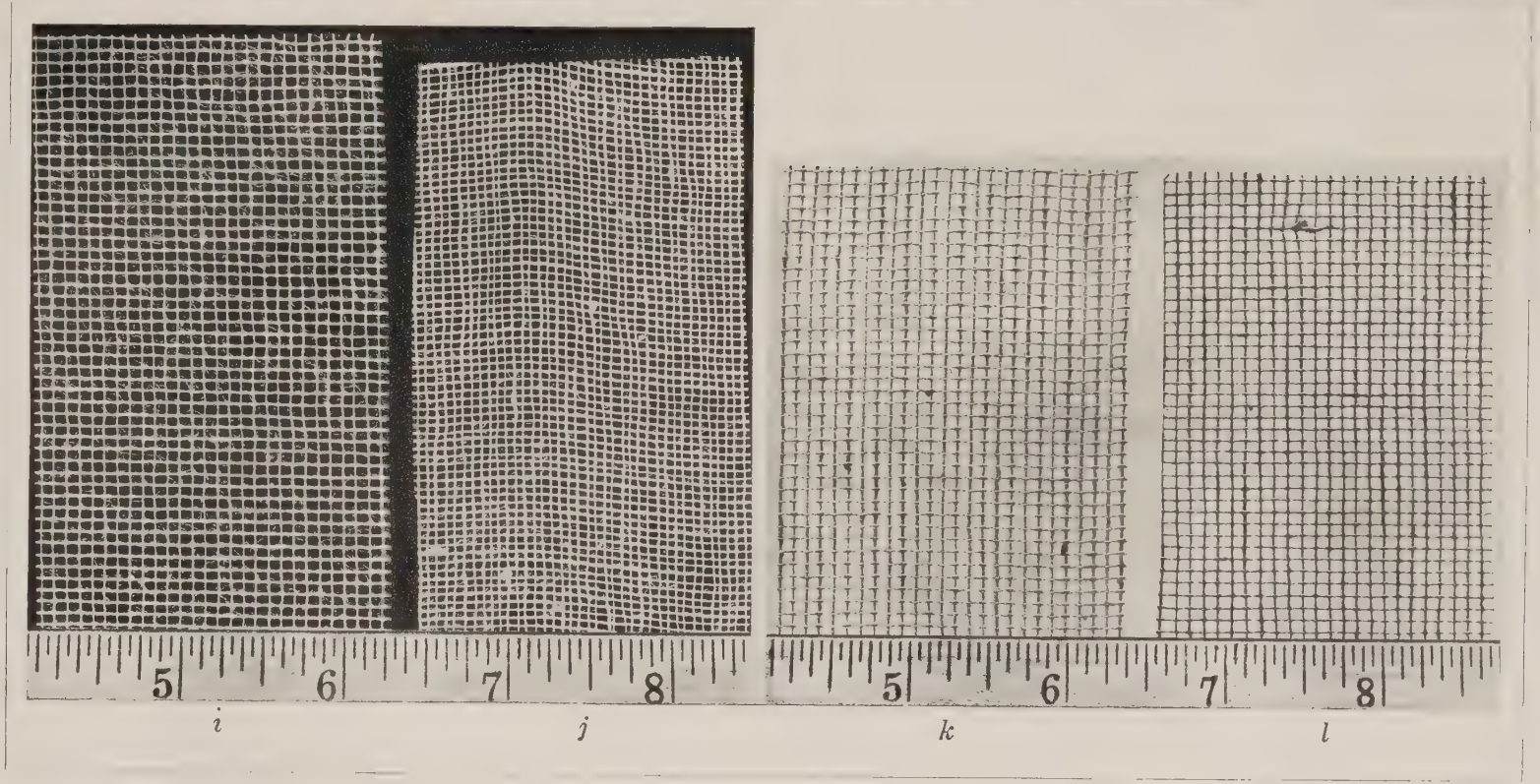

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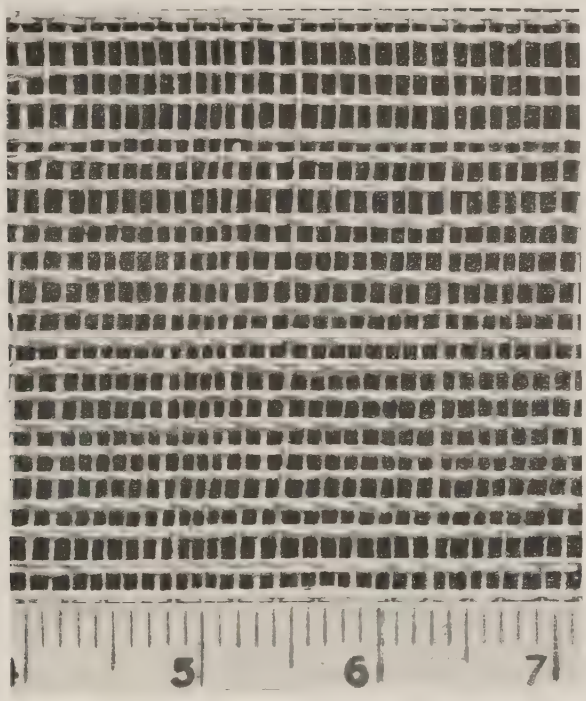

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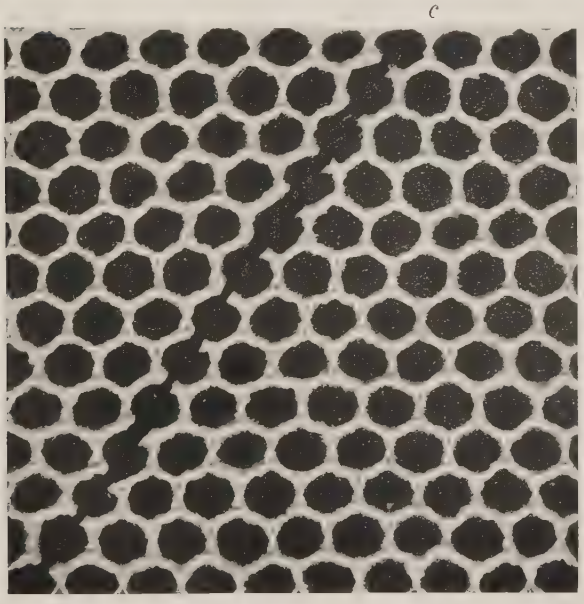

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PLATE 6.

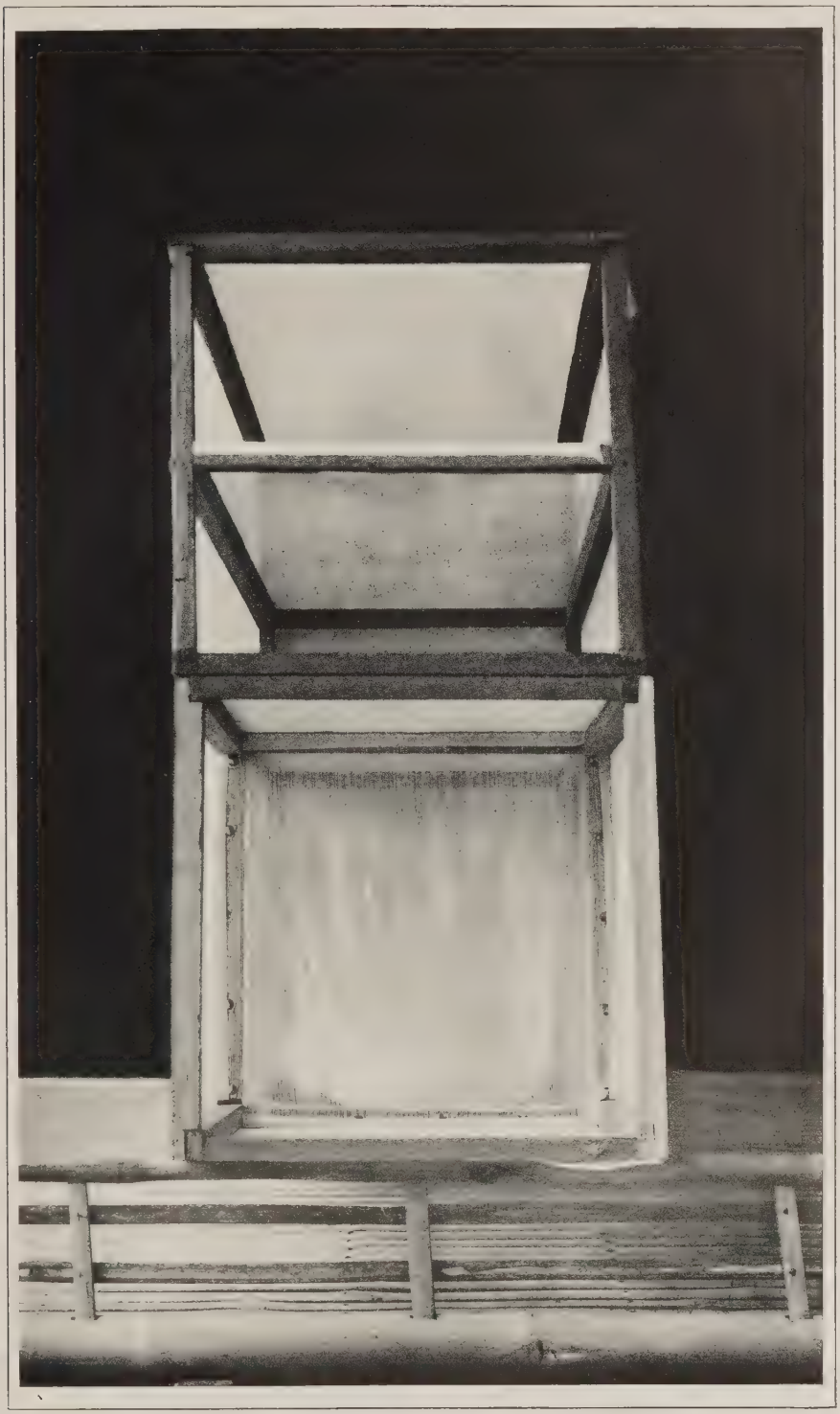

PLATE 7.

PLATE 8.

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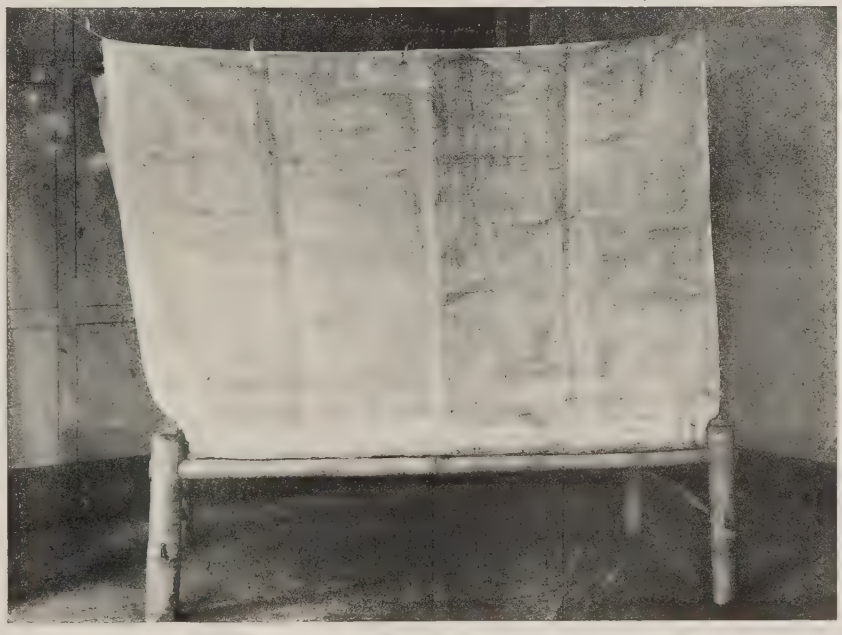

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PLATE 9.

PLATE 10.

OBSERVATIONS ON THE BONES OF NATIVE HORSES AFFECTED WITH OSTEOMALACIA

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FOUR PLATES AND TWO TEXT FIGURES

INTRODUCTION

Osteomalacia, popularly known as osteoporosis or "bighead" among horse owners, is a disease of metabolism characterized principally by the softening and porosity of bones. Nothing definite is known of its etiology. Different theories have been advanced at various times, but for the sake of brevity no attempt will be made here to explain them further, for they have been thoroughly and critically discussed by Kintner and Holt(6) in their recent paper on this equine disease.

What condition or factor brings about pathologic changes in the bones has likewise been a subject of great speculation. According to Hess,(3) Virchow in 1853 advanced the theory that in osteomalacia the lesions are due to increased absorption of calcarous substance, and Pommer in 1885 claimed that they are brought about by decreased calcification. Hess also makes reference to von Ricklinghausen who was of the opinion that "halisteresis," bone dissolution, is the condition responsible for the changes, and to Schmorl and Looser who claimed that they are due to failure of calcification. Wells(13) and Hutyra and Marek(4) seem to support the theory of Virchow that increased absorption of lime salts from the calcified tissue causes the softening or brittleness and rarefication of bones.

A survey of the available literature on the subject revealed that only Koster,(7) Gonzalez and Villegas,(2) and Kintner and Holt(6) have written on osteomalacia in local animals, in spite of the fact that this equine malady is common among native Philippine as well as imported horses and that it is of tremendous economic importance. In fact, at present this is one of the most important diseases that seriously hamper the work of the Government in the improvement of the native stock.

The present paper reports the writer's findings with respect to the changes in the bones of ten cases of osteomalacia in native horses that came under his direct observation from time to time during the period from 1924 to 1932. The study was undertaken primarily to determine whether or not the disease under local conditions develops the same pathologic changes in the bones as those observed in cases reported in other countries, and to ascertain what possible relation the disease has to various types of lameness among horses. It is also hoped that the data reported herein will be of some interest to veterinarians who seek an explanation for the frequent occurrence of fractures in horses, even from apparently very insignificant causes.

MATERIALS

Bones of ten native horses observed to be suffering from osteomalacia have been employed in the present study. In all the cases the bones of each individual animal were freed from other tissues, such as muscles, ligaments, fat, and bone marrow, by maceration. Two of the horses came from the hospital of the College of Veterinary Science, one was presented to the college by Mr. John Dumas, of Calauan, Laguna, three were used by the classes in surgical exercises, and four were obtained from the Department of Animal Husbandry, College of Agriculture. It might be mentioned here that the course and symptoms of the disease in the last four horses had been under the writer's observation ever since the first clinical symptoms were noted, and that, in addition, six other horses affected with this disease had been examined from time to time.

In order to facilitate the presentation and discussion of data herein recorded, the horses from the veterinary hospital are designated here as horses VH1 and VH2; that from Mr. Dumas, horse D3; those used by the classes in surgery, horses VS4, VS5, and VS6; and those from the animal-husbandry department, horses AH7, AH8, AH9, and AH10. A brief history of each animal, before it died or was sacrificed, follows:

Horse VH1.—Sex, stallion; color, bay; age, 5 years; height, 48 inches; use, carretela pony; place of origin, Los Baños, Laguna. This horse was an in-case in the Veterinary Hospital at one time, being treated for chronic nasal catarrh. After one week in the hospital the animal developed pneumonia from which he died about six or eight days later. When the animal was first brought in, he was also observed to be suffering from osteoporosis as indicated by the slight bulging of the face and moderately thickened lower jaw.

Horse VH2.—Sex, stallion; color, blood bay; age, 8 years; height, 49 inches; use, carromata pony; place of origin, Bay, Laguna. This animal was entered as a case of lameness due to bilateral spavin, but because of the fact that the animal did not respond favorably to the treatment given by the hospital, the owner finally decided to sell the animal to the College to be used by the Department of Anatomy for laboratory purposes. A thorough external examination performed by the writer before the animal was sacrificed, disclosed the following: The mandibular space was abnormally narrow due to the thickening of the rami of the mandible; there was palpated a nodular swelling at the medial aspect of the proximal third of the left metacarpus; slight elevation a little above the coronary region of the anterior limb; and swelling of the medial aspect of both hock joints.

Horse D3.—Sex, mare; color, dun; age, 6 years; height, 50 inches; use, for breeding; place of origin, Calauan, Laguna. The mare had been going lame in the posterior limb for some time before she was brought to the Veterinary Hospital for examination and treatment. She was found pregnant and suffering from some chronic hip trouble; the left gluteal region was already depressed due to the atrophy of the gluteal muscles. The condition being no longer amenable to treatment, the owner decided to donate the animal to the College for laboratory purposes. The only clinical symptom that led us to suspect that the mare was affected with osteomalacia consisted of slightly thickened and nodular lower jaw.

Horse VS4.—Sex, gelding; color, chestnut; age, 10 years; height, 48 inches; use, carromata pony; place of origin, Calamba, Laguna. This animal was bought by the College purposely for surgical exercises. He was lame in one of the posterior limbs. There was a slight swelling of the face. The mandible was somewhat thickened and the hock joints were slightly enlarged.

Horse VS5.—Sex, stallion; color, dark bay; age, 8 years; height, 49 inches; use, carromata pony; place of origin, San Pablo, Laguna. This animal presented a very typical picture of osteomalacia; the face was bulging and the lower jaw was very much thickened.

Horse VS6.—Sex, stallion; color, iron gray; age, 12 years; height, 48 inches; use, carromata pony; place of origin, Bay, Laguna. The only features observed in this animal indicative of osteomalacia consisted of the markedly thickened rami of the mandible and the enlargement of the hock joints.

Horse AH7.—Sex, stallion; color, cream; age, 17 years; height, 50 inches; use, for breeding; place of origin, College Campus, Los Baños, Laguna. This was one of the first stallions of the Department of Animal Husbandry, College of Agriculture. At about 8 years of age the animal suffered from time to time from an intermittent lameness in the hind limbs, apparently due to spavin. The animal had to be sacrificed because of the complete fracture of one of the anterior cannon bones, as a result of jumping on the manger.

Horse AH8.—Sex, mare; color, chestnut; age, 11 years; height, 50 inches; use, for breeding; place of origin, College Campus, Los Baños, Laguna. When this animal was about four years old, her head started bulging in the facial region. At the time she was sacrificed, because of the complete fracture of one of the femurs, her head was considerably enlarged.

The animal had also been suffering from lameness in the hind limbs from time to time.

Horse AH9.—Sex, mare; color, bay; age, 15 years; height, 49 inches; use, for breeding; place of origin, College Campus, Los Baños, Laguna. This animal was one of the first mares of the Animal Husbandry Department, College of Agriculture. She died of dystokia.

Horse AH10.—Sex, stallion; color, iron gray; age, 14 years; height, 53 inches; use, for breeding; place of origin, College Campus, Los Baños, Laguna. During life the only clinical symptoms that led us to believe that the animal was affected with osteomalacia consisted of the slight thickening of the lower jaw. Paraplegia, induced by osteoporosis, and found to be due to the compression of the lumbar components of the lumbo-sacral plexus by exostoses in the lumbar vertebræ, was the immediate cause of death according to the report by San Agustin.(12)

TABLE 1.—*Showing the average weights of bones of the affected and control animals.*

Bones.	Average weight.		Excess in favor of control.	Difference.
	Osteomalacic.	Control.		
	g.	*g.*	*g.*	*Per cent.*
Skull (with mandible)	2,322.3	2,786.1	462.8	16.6
Fourth cervical vertebra	94	130.0	36.0	27.8
Ninth thoracic vertebra	29.5	46.9	17.4	37.1
Third lumbar vertebra	42.8	62.9	20.1	31.9
Sacrum	115.6	168.8	53.2	31.5
Ninth rib	41.9	59.1	10.2	19.5
Scapula	234.3	310.6	76.3	24.5
Humerus	438.0	502.2	64.2	12.7
Radius and ulna	414.7	481.4	66.7	13.8
Third metacarpal	155.6	217.6	62.0	28.4
First phalanx (anterior limb)	61.1	67.1	6.0	8.9
Ossa coxarum	735.8	867.6	131.8	15.1
Femur	621.0	717.3	96.3	13.4
Tibia	422.6	512.4	89.8	17.5
Third metacarpal	212.7	259.7	47.0	18.0
First phalanx (posterior limb)	57.8	62.1	4.3	6.9

WEIGHT OF THE BONES

After the bones of each horse had been perfectly macerated and dried, the weights of the skull, the long and flat bones from both anterior and posterior extremities, one representative from each region of the vertebral column, except the coccygeal, and from the ribs, were determined and compared with those of the normal animals. This was undertaken for the purpose of gaining some information concerning the effect of the disease upon the weights of the bones. In the selection of bones from a normal animal as control, care was taken to use bones from an

animal of practically the same age and height as the one under study. The nature of the teeth was used in the determination of age, and the length of the long bones of the extremities was used as a criterion for comparing height. In Table 1 the average weights of the various bones of the osteomalacic and the control animals are compared and the percentages of difference given.

As may be noticed from Table 1 the bones of the osteomalacic animals are invariably lighter than the corresponding bones of the control. The difference, however, is not at all uniform in the same bones in different animals nor in various bones in the same animal. It seems, however, that the bones of the vertebral column and the flat bones are in general the worst affected in the decrease in weight, and that the decrease is dependent upon the pathologic lesions in the bones. It is interesting to note also that even the abnormally enlarged skulls in some cases were much diminished in weight.

ANATOMICAL CHANGES IN THE BONES

The macerated bones of each osteomalacic animal were carefully examined. The following is the record of the individual animal:

Horse VH1.—The face was slightly bulging. There was a marked porosity of the outer cortex of the bones of both face and the cranium, especially the frontal and maxillæ. Attached to the surface of the bones were thin plates of bony tissue which were scalelike and could be easily peeled off. The maxillary tuberosity was very much enlarged and brittle. The ventral border of the rami of the mandible was nodular and slightly thickened; porosity was relatively marked in the nodular parts. Throughout the vertebral column the rarefication of the outer cortex of the bones was very manifest. This was also true in the case of the ribs. Other than the porosity of the scapulae, the pelvic bones, and the tuberosities and other eminences of the long bones, the bones of the thoracic and pelvic limbs showed no particular features.

Horse VH2.—The rami of the mandible were abnormally thick, especially along the ventral border. There was a generalized porosity of the bones of the head, in spite of the absence of the swelling of the face. Scalelike pieces of bones were abundant. The vertebræ, the ribs, and the flat bones of the anterior and posterior limbs were likewise porous. The compact substance of the long bones of the extremities seemed to be not very much affected, the porosity being confined in the eminences. The second and the third metacarpal bones on the left side were completely fused, and at their proximal third there was an exostosis. The distal extremity of one of the first phalanges and the proximal part of one of the second phalanges presented in front irregular bony growth. The tarsal bones, with the exception of the tibial and fibular, were consolidated in both sides and were fused with the metatarsal bones.

Horse D3.—There was a slight porosity of the bones of the skull and the rami of the mandible were abnormally thick along the ventral border. With the exception of the presence of exostosis in the lateral aspect of the superior ischiatic spine and the adjacent part of the shaft of the ilium and the lateral margin of the acetabulum, the rest of the bones of this animal appeared to be normal.

Horse VS4.—Other than the presence of exostosis in the proximal part of the anteromedial aspect of the large metatarsal bones in both sides, the bone lesions presented by this case were practically similar to those of horse VH1.

Horse VS5.—There was a moderate swelling of the face. Marked porosity was observed throughout the skull. The intermandibular space was narrow due to the thickened rami of the mandible. The ribs and vertebræ showed only slight porosity, and the bones of the extremities appeared to be not yet affected.

Horse VS6.—In spite of the absence of the swelling of the face and marked deformity of the mandible, the bones of this animal, including the long bones of the limbs, were all badly affected, the rarefication being relatively severe in the bones of the face, vertebral column, ribs, and flat bones of the limbs. Exostosis was present in the anteromedial aspect of the proximal extremities of both large metatarsal bones.

Horse AH7.—The size of the skull was apparently normal, no deformity being observed except the moderately thickened rami of the mandible. All the bones of the face and cranium were very porous and rough and there were plenty of small indentations. The lesions were most salient along the ventral borders of the rami of the mandible, in the crests which limit the temporal fossa, and in the supraorbital and coronoid processes and the zygomatic arches. The free end of the nasal bones was very brittle. The bones of the vertebral column and the ribs were almost spongy in nature. The spinous processes of most of the thoracic and lumbar vertebræ and the sacrum were very much thickened and deformed. The fifth and sixth lumbar vertebræ were consolidated. The last four pairs of ribs were abnormally twisted and they were studded with irregular eminences or tubercles on their costal surface. All the bones of the anterior and posterior limbs also showed marked porosity, though they were not uniformly affected. The large metacarpals were bowed, and the fourth and central, the third and the first and second tarsals in both limbs were completely fused.

Horse AH8.—Of all the cases studied this animal was worst affected. The skull was considerably deformed, having a very pronounced external swelling of the maxillary, malar, and lacrimal bones, and abnormally thickened rami of the mandible. All bones of the face and cranium were fragile and could easily be crushed into powder, even with the fingers. The external surface of the bones presented numerous irregular depressions. The nasal cavity was narrow and the infraorbital foramen as well as the mental foramen was somewhat obliterated. The intermandibular space was very narrow and the swelling of the rami of the mandible was much more pronounced along the alveolar border than along the ventral border. All the alveolar cavities of both the upper and the lower jaws were very much widened. The ribs and the vertebræ were weak and fragile and almost spongy in character. All the bones of both the

anterior and the posterior limbs were conspicuously porous, especially the scapulæ, the pelvic bones, the humerus, and the femurs. Both radii were abnormally bowed. The first phalanges of the anterior limbs presented exostosis on either side midway between the proximal and distal extremities. With the exception of the tibial and fibular tarsal bones all the bones of the tarsus on both sides were fused.

Horse AH9.—There was no enlargement of the face, but all the bones of the skull were weak and porous. The external surface of the maxillary bones presented numerous irregularly excavated areas. The nasal bones were almost transparent. The mandible presented only a slight swelling along the alveolar border of the rami. All the teeth were very loose because of the enlarged alveolar cavities. The vertebræ in all the regions of the vertebral column as well as the ribs were almost spongy in nature. The bones of both the anterior and posterior limbs seemed to be not very much affected, showing only slight porosity of the external cortex.

Horse AH10.—There was a generalized porosity of the skull. The mandible was slightly thickened and was characterized by the presence of bony protuberances on the lateral surface of the alveolar border of the rami. The mental foramen was somewhat obliterated and overhung by exostoses. The ribs and the bones of the vertebral column were all porous and weak; the spinous processes of the thoracic and lumbar vertebræ were more or less spongy in nature. On the ventral surface of the bodies of the third, fourth, and fifth lumbar vertebræ large exostoses were found, practically obliterated the intervertebral foramina formed by these vertebræ. The bones of the anterior and posterior extremities likewise showed marked porosity. The tuber scapulæ and the coronoid processes were abnormally large and irregular because of the presence of exostosis. The large and small metacarpals in both limbs were completely fused. There was also exostosis in the proximal part of the dorsal surface of the first phalanx of one of the anterior limbs, and on the lateral surface of the right superior ischiatic spine just a little above the rim of the acetabulum. The central and the third tarsal bones were consolidated; and the fourth and the fused first and second tarsal bones were very much enlarged and deformed because of the presence of exostosis.

Based on the summary of the various features observed in the skeletons of the animals studied, it may be inferred that in general the anatomical changes in the bones of native horses suffering from osteomalacia are similar to those described by other investigators. It seems also safe to state here that neither the breed of animal nor the environmental conditions—climatic or otherwise—have any influence upon the macroscopic picture of the bones of animal affected with this disease.

Kintner and Holt(6) say that in imported army horses and mules in the Philippines "all the bones of the skeleton are involved in the process, and that the variation in the degree of severity is due, probably, to external influences, such as mechanical irritation, etc." The present study, however, inclines the writer to believe that, although all the bones are involved, the

rarefication of the external cortex does not occur throughout the bones of the skeleton at the same time. In the incipient stage of the disease the gross changes are invariably confined to the bones of the skull, and only in severe cases do the long bones of the extremities show external lesions. This condition may be accounted for by the fact that, according to Friedberger and Frohner,(1) the decalcification of bones starts from within outward. Thus, it is but natural to expect that bones having thin cortex or compact substance should suffer first. Careful study of the bones of incipient or mild cases, moderate cases, and severe cases has convinced the writer that rarefication of bones in osteomalacia manifests itself externally in the following order: (*a*) Skull, (*b*) vertebræ and ribs, (*c*) flat bones of the extremities, like scapula and os coxæ, and (*d*) long bones of the extremities.

The bilateral enlargement or bulging of the skull in the region of the face is not always a constant feature in osteomalacia. The writer has observed that such deformity of the skull develops only when the animal becomes affected with the disease while still young. Niimi(10) also seems to attribute the slightness of the swelling of the facial bones of the horses he used in his experimental studies on osteomalacia to old age. Other deformities of the skeleton, like exostosis, abnormal bending of long bones and ribs, fusion of the vertebræ and the tarsal bones, etc., are only secondary in nature, and they are probably due to external influences such as blows, kicks, and other forms of mechanical irritation. It has been observed that, except for the enlargement of the skull in some cases, the size of the rest of the bones of the skeleton was not affected by the disease, even in very severe cases where the gross lesions were generalized, and that in severe cases the medullary cavity of the long bones was invariably enlarged.

Because of the common occurrence of exostoses and other skeletal deformities in affected animals, the origin and cause of certain types of lameness among horses may be traced to osteomalacia. In the majority of the cases that the writer has observed, lameness in either the fore or hind limbs constituted the commonest complaint.

HISTOLOGY OF THE COMPACT SUBSTANCE OF LONG BONES

To determine what effect the disease has upon the lamellar systems of the compact substance of osseous tissue, several transversely ground sections from the middle of the shaft of the

large metacarpal of horse AH7 and the femur of horse AH8 were prepared and mounted in balsam. It may be mentioned

FIG. 1. Segment of transversely ground section through the middle of shaft of the femur of horse AH8 as seen under Spencer dissecting microscope No. 80. Stained black with silver nitrate solution. *a*, Outer, or periosteal, surface; *b*, inner, or medullary, surface; *c*, bits of bony tissue.

here that the bones of these animals were selected because, besides having generalized gross changes, they were the ones

sacrificed due to complete fracture of the said bones. Similar sections were also prepared from the same bones of their respective control. Some of the sections were stained with 1 per cent silver nitrate solution.

Careful comparative study under the microscope of both the sections of osteomalacic specimens and those prepared from the same bones of the control animals disclosed the following features: The thickness of the compact substance of the shaft of long bones studied has been greatly diminished; presence of numerous irregular bits of bony tissue (fig. 1) attached to the inner or medullary surface; presence of erosions and small excavations on the external or periosteal surface; both the periosteal lamellæ and the endosteal lamellæ have been considerably decreased in thickness and in some places they are totally absent; and most of the Haversian canals, which appear in the sections as irregular empty spaces of different sizes (fig. 2), have been amply enlarged, and the surrounding concentric lamellæ, in many places, are no longer present. Very likely many of the large spaces have been due to the coalescence of the adjacent enlarged Haversian canals. With this microscopic picture of the changes in the lamellar systems it may be assumed that the destructive elements of the calcarous tissue—osteoclasts or otherwise—in the compact substance of the long bones are mostly lodged in the Haversian canals.

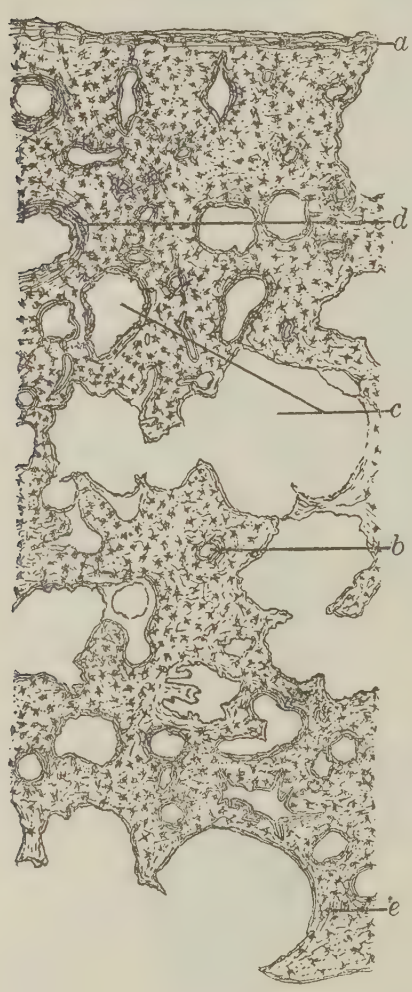

FIG. 2. Segment of transversely ground section through the middle of the large metacarpal bone of horse AH7, drawn under Reichart microscope with ocular No. 1 (13x) and objective No. 3 (10x). *a*, Outer circumferential, or periosteal, lamellæ; *b*, normal Haversian canal; *c*, enlarged Haversian canals; *d*, concentric lamellæ; *e*, inner circumferential, or endosteal, lamellæ.

BREAKING STRENGTH OF LONG BONES

Comparative tests were conducted to determine the weights required to cause fracture in different long bones of the normal and the osteomalacic animals. The Tinus Olsen testing machine of the Bureau of Science was employed in the tests. Each specimen was arranged in such a way that the span between its stationary supports was exactly 15 centimeters and that the mechanical weight was gradually applied midway between the supports.

TABLE 2.—*Showing the average weights required to cause fracture in the long bones of control and osteomalacic animals.*

Name of bone.	Control.	Osteomalacic.	Excess in favor of control.	Difference.
	kg.	*kg.*	*kg.*	*Per cent.*
Humerus	1,702	1,170	532	31
Radius	2,032	1,505	527	25
Large metacarpal	1,777	1,237	540	29
First phalanx	5,750	2,285	3,465	60
Femur	1,640	1,375	265	16
Tibia	2,620	1,502	1,118	42
Large metatarsal	2,610	1,647	963	36
Ninth rib	200	120	78	39

As may be noted from Table 2 this experiment demonstrated that the weights required to cause fracture in various long bones of both the normal and the osteomalacic native horses vary with the kind of bone, and that under the same conditions bones from horses affected with osteomalacic fracture under less weight than do bones from normal animals. In the last column of the table are given the average percentages of the difference, and, as may be noticed here, the percentages vary in different bones.

CHANGES IN THE CHEMICAL COMPOSITION OF BONES

Available data concerning the chemical changes occurring in the bones of horses affected with osteomalacia are not at all uniform. According to Friedberger and Frohner(1) the lime content of osteomalacic bones is reduced more than one-half and the water content increased. McCrudden(8) observed a decrease in calcium and phosphorus and an increase in magnesium and sulphur in the ribs of two horses affected with the disease. Mohler(9) claims that bones of osteomalacic animals contain less fat, phosphoric acid, lime, and soda and more organic matter

and silicic acid. Ingle,(5) in his comparative analysis of the bones of diseased and normal horses, mules, and donkeys, found little difference in the composition of bones of osteomalacic animals as compared with those of the normal ones, and in normal bones he observed larger proportions of ash, lime, and phosphoric acid. Kintner and Holt(6) state that there is a reduction of the total mineral content in the bones of affected animals, and that the percentage of calcium and phosphorus in the bone ash of the affected animals is approximately the same as in the normal ones, but the magnesium content is much increased. Niimi and Aoki,(11) in their chemical analysis of the ribs of osteomalacic cases produced experimentally, also did not obtain uniform results. In one group of horses they found a reduction in water and an increase in calcium and phosphoric acid, and in another group there was more water and less ash. The quantity of magnesium also was not the same in the two groups.

TABLE 3.—*Showing the average percentages of the chemical constituents of dried macerated bones from osteomalacic and normal horses.*

Bones.	Subject.	Moisture (H_2O).	Fat.	Phosphate of lime ($Ca_3(PO_4)_2$).	Carbonate of lime ($CaCO_3$).	Phosphate of magnesia (Mg_3PO_4).	Carbonate of sodium (Na_2CO_3).	Chloride of sodium (NaCl).
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Humerus	Control	7.34	0.55	31.91	6.92	2.16	7.34	0.10
Do.	Affected	8.70	1.68	41.66	4.72	2.68	4.79	0.15
Radius and ulna	Control	7.44	0.93	44.81	8.37	3.74	8.86	0.45
Do.	Affected	7.50	0.81	47.22	6.71	2.51	7.11	0.36
Metacarpal	Control	7.39	0.99	31.00	7.43	2.13	7.86	0.44
Do.	Affected	8.34	1.34	32.02	4.28	2.36	4.48	0.16
Femur	Control	7.63	1.14	42.89	8.04	1.08	8.78	0.18
Do.	Affected	6.91	1.26	38.31	5.90	2.82	6.25	0.20
Tibia	Control	8.23	0.53	25.63	10.55	5.10	11.18	0.27
Do.	Affected	7.41	1.39	21.32	7.12	1.65	7.48	0.05
Metatarsal	Control	8.71	0.83	24.64	8.39	2.97	8.90	0.05
Do.	Affected	8.49	1.16	39.18	6.10	2.27	6.46	0.32
Vertebræ	Control	7.64	1.83	41.10	10.04	2.13	10.49	0.06
Do.	Affected	8.43	0.73	25.18	6.99	1.73	7.41	0.42
Ribs	Control	8.09	1.54	38.81	7.29	3.42	11.20	0.37
Do.	Affected	7.98	0.93	32.15	5.72	3.11	6.07	0.34
Total average	Control	7.80	1.04	35.09	8.37	2.84	9.32	0.24
Do.	Affected	7.98	1.03	34.63	5.94	2.39	6.25	0.25

In order to gain some information concerning the changes in the chemical composition of bones of native horses affected with

osteomalacia, chemical analysis * of bones from affected and normal animals was undertaken. Samples for anlysis were prepared from different bones by filing with a rasp.

The analyses in Table 3 show that the different chemical constituents of dried macerated bones from normal as well as from osteomalacic native horses vary in different bones, and that an osteomalacic specimen is not always deficient in certain chemical elements, as compared with the corresponding specimen from a normal animal.

The percentage of moisture and fat is approximately the same for osteomalacic as for normal bones. The figures given here, of course, do not represent the total percentage of these elements in fresh bones. The total average percentages obtained for the different principal inorganic constituents of bones are as follows: For normal bones, phosphate of lime, 35.09; carbonate of lime, 8.37; phosphate of magnesium, 2.84; carbonate of sodium, 9.32; and chloride of sodium, 0.24. For osteomalacic bones, phosphate of lime, 34.63; carbonate of lime, 5.94; carbonate of sodium, 6.25; and chloride of sodium, 0.25.

The average percentage obtained for the total inorganic constituents of bones is 55.86 for normal horses and 49.46 for osteomalacic animals, a difference of 6.40. This clearly shows that there is also a reduction of the total mineral content in the bones of native horses affected with osteomalacia; the reduction is principally noticed in the carbonate of lime, phosphate of lime, and carbonate of sodium. The phosphate of magnesium is slightly increased, but the chloride of sodium is practically the same as in normal bones.

SUMMARY AND CONCLUSIONS

1. Dried macerated bones of seven male and three female native Philippine horses affected with osteomalacia, ranging in age from 5 to 17 years, were used in the present study. For comparative purposes bones of normal native horses were also included in this investigation.

2. Comparative study of bones of osteomalacic and normal horses has demonstrated that the weight of bones of affected animals is invariably much diminished. The percentage of dif-

* The chemical analyses of bones were made possible through the courtesy of the Department of Chemistry, College of Agriculture, University of the Philippines. Grateful acknowledgment is made here to the members of the department, particularly to Mr. L. Yñalvez, who made the analyses, for their very valuable and kind coöperation.

ference, however, varies not only in different animals but also in different bones of the same animal, depending upon the severity of the gross lesions in the bones.

3. The macroscopic picture of the gross anatomical changes in the bones of native horses suffering from osteomalacia fairly agrees with what has been previously described by other investigators. Environmental conditions and breed of animals seem to play no rôle in the anatomical changes of the diseased bones.

4. It has been observed that rarefication of the cortex or compact substances of bones does not occur throughout the bones of the skeleton at the same time, but is confined to the bones of the skull in the incipient stage; other bones, especially those of the extremities, are affected only in severe cases.

5. The bilateral swelling of the skull in the region of the maxillary, lacrimal, and malar bones has not been noted as a constant feature in horses affected with osteomalacia. The results of the present investigation prompt the writer to believe that animals already advanced in age when affected with this disease, do not develop such a deformity. The mandible on the other hand has been observed in all cases studied to have increased in thickness in varying degree, either along the ventral or the alveolar border of the rami. In no case have the rest of the bones of the skeleton been found to have changed in size, except where exostoses were present.

6. The thickness of the compact substance of the shaft of the long bones of affected animals is much diminished. This condition may be accounted for by the destruction of the inner and the outer circumferential lamellæ. The Haversian canals are very much enlarged and the concentric lamellæ surrounding them are totally absent in many places.

7. Bones from osteomalacic horses have been found to require less weight to fracture them than bones from normal animals. The required weights, however, vary in different animals and in different bones of the same animal.

8. A reduction of the total mineral content has also been observed in the bones of native horses affected with osteomalacia. An average of 55.86 per cent for normal horses and 49.46 per cent for affected animals has been found to constitute the total inorganic constituent. The reduction was noted particularly in carbonate of lime, phosphate of lime, and carbonate of sodium.

9. Many cases of lameness among horses, especially those of obscure origin, may be attributed to osteomalacia, exostoses,

anchyloses, and other skeletal defects very common in the bones of animals affected with this disease.

10. The frequent occurrence of fractures in osteomalacic animals, even from very insignificant causes, must be due to the decrease in strength of the bones because of the diminished thickness and the porosity of the compact substance.

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ILLUSTRATIONS

PLATE 1

FIG. 1. A case of osteomalacia in native horse AH8, showing the typical bilateral swelling of the face.

FIGS. 2 and 3. Skull of an osteomalacic horse at incipient stage. Note the erosions on the surface of the skull and the nodular thickenings of the ventral borders of the rami of the mandible.

PLATE 2

Some bones of a very severe case of osteomalacia (horse AH7), showing no bulging of the face and generalized porosity.

PLATE 3

Dorsal view of the skull and ventral view of the mandible of horse AH8. Note the marked swelling of the face and the abnormally thickened rami of the mandible.

PLATE 4

The skull of horse AH8 showing the enlarged alveolar cavities and partly destroyed interalveolar septa.

TEXT FIGURES

FIG. 1. Segment of transversely ground section through the middle of shaft of the femur of horse AH8 as seen under Spenser dissecting microscope No. 80. Stained black with silver nitrate solution. *a*, Outer, or periosteal, surface; *b*, inner, or medullary, surface; *c*, bits of bony tissue.

2. Segment of transversely ground section through the middle of the large metacarpal bone of horse AH7, drawn under Reichart microscope with ocular No. 1 (13x) and objective No. 3 (10x). *a*, Outer circumferential, or periosteal, lamellæ; *b*, normal Haversian canal; *c*, enlarged Haversian canals; *d*, concentric lamellæ; *e*, inner circumferential, or endosteal, lamellæ.

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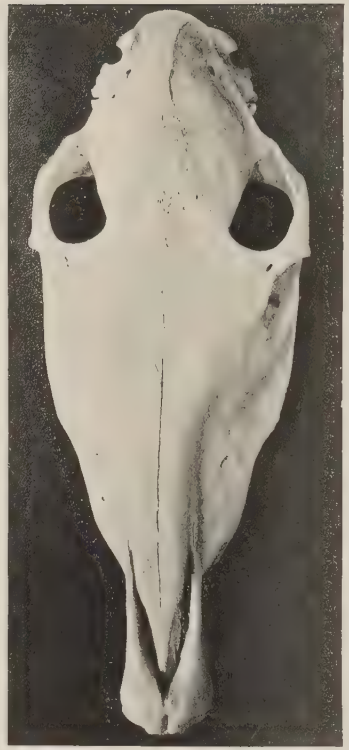

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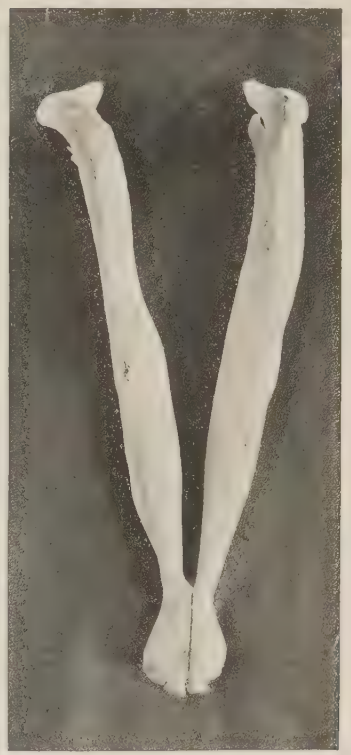

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PLATE 1.

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PLATE 2.

PLATE 3.

PLATE 4.

A FATAL CASE OF NONDYSENTERIC AMŒBIASIS

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THREE PLATES

INTRODUCTION

Dysentery has been defined as a symptom-complex consisting of frequent bowel movements, bloody stools, and tenesmus; and ever since Musgrave(6) coined the word "amebiasis," in 1904, a laboratory report of the presence of *Entamœba histolytica* in a stool invariably brings to mind that classical triad of complaints.

However, observations within the last thirty years have brought to light variable types of clinical manifestations other than dysentery when this protozoan parasite gains entrance into the human body. Musgrave,(7) in 1910, described "intestinal amebiasis without diarrhea," and Walker,(10) in 1913, classified carriers in this disease as convalescent and contact carriers. Reed,(8) in 1922, introduced into the nomenclature the term "non-dysenteric amebiasis" to signify conditions in which dysentery does not occur concomitantly with the presence of amœbæ, as in carriers and in the possible but still doubtful amœbiasis of the bones in arthritis deformans, Ely's second great type of arthritis.

The present case is reported for what it may be worth in the study of extra-intestinal infections, it being unquestionably of a nondysenteric type (carrier) but with the sequelæ of abscess formation in the liver, kidney, lungs, and brain.

A detailed résumé of the hospital and autopsy records of the case are herein presented together with the microscopic findings to show its beginning, course, and fatal end.

CLINICAL RECORD; CASE 61392

General data.—R. L., 70-year-old male Filipino, born in Bulacan, residing in Manila, laborer, came to the Philippine General Hospital June 25, 1918, complaining of abdominal pain of three weeks' duration.

Habits.—Used to drink wine (ginebra) and tuba; smokes a little.

Family history.—Father died of paralysis of half of the body at the age of 70 years. Mother died of a disease unknown to the patient. One sister died of cholera. Has one brother living and well.

Previous illness.—Had a fall at the age of 7 years, as a result of which patient was unconscious for two days. At the age of 12, he had another fall which caused dislocation of the right shoulder. At the age of 25, he had "*pasma*," as a result of which the right half of his face became paralyzed.

Present illness.—Began three weeks ago with inability of the patient to move his bowels. For this he went to the dispensary of the Philippine General Hospital where he received treatment. He got well apparently after two days, but later he felt pain in the abdomen. For this he was treated by a physician, but there was no relief. So he applied for admission into the hospital.

Present condition.—Patient is complaining of severe abdominal pain. Walks with difficulty. Prefers lying in bed.

Physical examination.—Patient is a fairly developed and fairly nourished old man, able to sit up and stand but prefers to remain in bed most of the time. Face is asymmetrical. Right half of face unable to contract. Right eye does not close very well. Nose drawn towards the left. Left angle of mouth drawn upward towards the left. Right angle of mouth drawn downward. Teeth are poorly kept. Tongue coated. No palpable masses in the neck, but pulsation is marked on each side. Chest is flat. Supraclavicular fossæ are much depressed. Expansion is very poor.

Palpation.—Increase of tactile fremitus over the apices, on the left interscapular area, and just below the inferior angle of left scapula.

Percussion.—Dullness over and around the apices, especially over the scapular regions. Dullness over the left axillary region and just below the inferior angle of left scapula.

Auscultation.—Respiratory murmur very faint throughout the chest. Respiration bronchial in character, particularly over the apices and the region below the inferior angle of left scapula. Crepitant râles heard over the same region.

Circulatory system.—Cardiac area of dullness not enlarged. Apex beat in the fifth interspace along the midclavicular line. Heartbeat normal in rate and rhythm.

Digestive system.—Tongue coated; appetite good; bowels constipated.

Abdominal examination.—Abdomen bulging on the right side. There is a palpable mass below the right subcostal margin. This mass is firm and connected or continuous with the liver and gives a dull resonance on percussion. The upper border of the liver is at the fifth rib in front; at the sixth interspace in the axillary area, and at the seventh interspace behind along the scapular line.

Genitourinary system.—Apparently normal.

Integumentary system.—The entire skin is pigmented and of an ashy color giving the appearance of bronze.

Muscular system.—Musculature rather thin and emaciated.

Clinical diagnosis.—Facial paralysis, right side. Abscess of the liver. Pulmonary tuberculosis.

OBSERVATIONS AND TREATMENT

Fæces examination was requested once and was negative. Blood examination showed a leucocyte count of 10,000 with a differential of 78 per cent polymorphonuclear neutophiles and 22 per cent of lymphocytes. X-ray showed a general enlargement of the liver and spondylitis deformans of the last dorsal and lumbar vertebræ. July 1, six days after admission, an exploratory puncture was made into the liver. Incision and drainage of the liver abscess were performed July 2. During the twenty-eight days after the operation, the wound was dressed daily and irrigation of the abscess with a solution of quinine bisulphate was made daily. Pain around the operated part was the constant complaint. Cough was persistent so that the giving of bromoform mixture and cresote inhalation were part of the treatment. One cubic centimeter of sodium cacodylate (10 per cent solution) was administered hypodermically once a day to strengthen the patient. Constipation, which was the principal complaint on admission, persisted as a troublesome feature of his illness and the physician in charge ordered cathartic enemata of saturated solution of magnesium sulphate on July 8, 10, and 21.

The patient died July 31, 1918, after thirty-six days in the hospital, with a temperature of 37° C. Fever was of the irregular type. The patient entered the hospital with a subnormal temperature, 36° C. After five days of apyrexia, the temperature rose to 38° C.; it fell to normal and subnormal on several occasions and rose to 38° C. at various times.

AUTOPSY RECORD; NECROPSY 6276

By Dr. C. MANALANG

Body is that of a well-developed and rather poorly nourished male adult Filipino, 70 years old, 32.50 kilograms in weight and 153 centimeters in length. Post-mortem rigidity slight; lividity slightly present on dependent portions. On the right upper quadrant of the abdomen is a vertical incision scar 10 centimeters in length with its upper extremity at the costal margin. A small strip of gauze is inserted through an opening 1 centimeter in diameter located at the seventh right interspace, 3 centimeters anterior of the right midaxillary line. There are no skin lesions.

On section in the subcutaneous tissue below the previously described scar are sutures. The anterior abdominal wall is adherent to the surface of the left lobe of the liver by means of firm, fibrous bands. The border of the liver extends 5 centimeters below the costal margin. Appendix is normal. The mesenteric glands are not enlarged. The convex surface of the right

lobe of the liver is completely obliterated by adhesions. The lateral surface of the left lung is free, and the right is adherent. The base of each lung is firmly adherent to the diaphragm. The thymus is replaced by fatty tissue. The pericardium contains normal fluid. A strong band of adhesion binds the posterior surface of the left ventricle to the pericardium.

The heart weighs 232 grams. The epicardium is thickened around the adhesion. The right ventricle contains a small amount of clot. Valves normal. The musculature is dark brown, firm, and normal in thickness. The base of the aorta is quite smooth.

The left lung is voluminous and crepitant except a slight partial consolidation of the posterior portion. Cut surface is dark gray, spongy, and very moist with frothy fluid. The consolidated area shows small portions which apparently contain air. The right lung is not removed as a whole, the inferior lobe being left and removed subsequently with the liver. Section of the right lung did not show any gross lesions in the nature of tubercles or caseation.

The spleen weights 77 grams, normal.

The left adrenal weighs 55 grams, normal.

The left kidney weighs 127 grams, cut surface is pale and swollen, the cortex thick. The capsule strips with resistance. The right adrenal and kidney which is smaller than the left are removed with the liver.

The stomach contains a small amount of thick yellowish material. The mucosa is intact.

The small intestine contains a dozen large ascarids. The mucosa is intact. The Peyer's patches in the lower ileum are pigmented but not enlarged. The large intestine contains formed, dark fæces. The mucosa is smooth and pale. No scars or ulcers are visible. Only minute pin-head erosions are visible in the cæcum.

The pancreas weighs 71 grams, normal.

The liver is about normal in size, and the previously described opening on the right side communicates with a cavity in the right lobe. This cavity is located on the posterior and superior portion of the lobe. It extends below the diaphragm, behind the liver, and involves the upper pole of the right kidney. It holds about 300 cubic centimeters of thick, ropy, creamy, and odorless material. Through the diaphragm it communicates with a similar cavity in the lung about 10 centimeters in diameter. The liver tissue surrounding the cavity is firm and infiltrated with white fibrous tissue. The cavity in the lung is surrounded by a firm, white wall, 5 centimeters in thickness. The inner surface of the cavity is lined by a white, soft, granular substance. The diaphragm is thick.

The bile ducts and gall bladder are normal.

The genitalia are normal.

The aorta shows irregular, hard, yellowish plaques.

The head. On removal of the skull, scalp, and dura matter, the anterior portion of the right frontal lobe of the brain is adherent to the dura matter and on removal a cavity the size of a hen's egg is exposed. This cavity is filled with ropy, thick, creamy, blood-streaked material. The surrounding brain is soft and blood tinged. Similar material infiltrates the subarachnoid space covering the inferior surface of the cerebellum. All the basal blood vessels show marked calcification of their walls.

Anatomic diagnosis: Amœbic abscess of the liver, lung, kidney, and brain; generalized arteriosclerosis; bronchopneumonia patch in the left lung; ascariasis.

GENERAL DISCUSSION

A study of the material on hand reveals the presence of abscesses in the liver, lungs, kidney, and brain, respectively. In all of these lesions, the trophozoites of *Entamœba histolytica* have been found. It is unfortunate that the part of the intestine which grossly showed minute superficial ulcerations is not available. Luckily, smears made during the autopsy from around the ulcers are available and are positive for both the trophozoites and cysts of the parasite. Without a doubt the course of infection could be traced from the intestinal lesions, through the portal circulation into the liver, forming an abscess therein. By contiguity the infection extended to the kidney. The lung abscess might have been produced either by contiguity from that of the liver or through the circulation. The brain abscess is probably metastatic from the liver.

ABSCESS OF THE BRAIN

The abscess of the brain is single and is located in the anterior portion of the right frontal lobe (Plate 1). It measures 9 centimeters in diameter. The wall of the abscess after the removal of the liquid contents shows a rough, finely nodular surface. Microscopically the amœbæ have been found to be numerous along the walls of the abscess. There is a distinct fibrous lining separating the healthy brain tissue from the necrosed substance of the wall of the cavity, beyond which no amœbæ were detected.

The formation of an amœbic abscess in the brain is extremely rare. In fact, Armitage,(1) who collected all the cases in the literature up to 1919, including his case, gives a total of 49. Armitage gives the geographic distribution of the forty-eight cases as follows: Madagascar, 1; Gulf of Mexico, 1; British India, 5; Indo-China, 7; Egypt, 24; Dutch Indies, 1; Tropical districts not precisely indicated, 2; France, 2; Germany, 1; England, 3.

To this list the present case is added. Armitage says:

> The statistics of the American commission to the Philippines did not record a single case in over 3,000 cases of liver abscess, and it is astonishing that in the rich Anglo-Indian and Franco-Algerian literature of the first half of the 19th Century only rare cases are found.

While I am certain that there has not been reported a case of amœbic brain abscess in the Philippine Islands, I am unable to verify the 3,000 liver abscess mentioned by Armitage. A minute search of the reports of the two American commissions, namely, that of the American Board for the study of Tropical Diseases headed by R. P. Strong, and that of the Johns Hopkins University Medical Commission to the Philippines, headed by L. B. Barker and S. Flexner, failed to confirm his statement. Dobell(4) writing on this point says:

> A good instance of confusion which is met with in the records of amœbiasis may be found in the published figures for the Philippines. We have numerous accounts of the prevalence of E. histolytica infections in the resident population, ranging from 0 to 70 per cent; and whilst the earlier records showed that amœbic dysentery and amœbiasis were responsible for a great part of the sickness and mortality in these islands, more modern and accurate investigation have shown that, although E. histolytica infections are very common, most of the dysentery is really bacillary.

ABSCESS OF THE LIVER, LUNG, AND KIDNEY

From the autopsy protocol it could be safely inferred that the abscess primarily developed in the liver. This has grown in size and by contiguity extended through the diaphragm, forming an abscess in the lower portion of the right lung and downwards to involve the upper pole of the right kidney. The liver, diaphragm, and surface of the right lobe of the lung are well adherent (Plates 2 and 3). The kidney with the abscess occurring superficially was subsequently separated from the adhesions to the liver. The walls of the abscess in the lung, liver, and kidney have practically the same gross appearance; that of the kidney (Plate 3), however, is somewhat nodular, while those of the lung and liver have a shaggy look. In all of these, microscopic examination shows a uniform tendency towards a walling off of the invading organism. There is formation of a more or less definite barrier of connective tissue with coincident inflammatory reaction products around the involved areas.

Rupture into the lung of an amœbic abscess of the liver has been mentioned by Strong(9) and Gilman(5) in their experience in the Philippines, but they have never encountered any kidney involvement.

Regarding liver abscesses occurring singly, Abriol(2) records among Filipinos 83 cases of amœbic colitis complicated by liver abscesses among 3,630 necropsies (1910–1917). Crowell(3) encountered 9 among 31 cases of amœbic colitis. Gilman(5) found 1 among 32 cases. Pains were taken to hunt for cysts of

amœbæ in all the abscesses encountered in the present case, but none was found. Exception, therefore, has to be taken to Abriol's statement that "Occasionally we may find lumps of translucent mucoid yellowish material which as a rule harbors encysted amœbæ."

THE CARRIER STATE

The evidence shows that this patient belongs to the group of "non-dysenteric amœbiasis" cases more commonly known as carriers. Smears from the intestinal contents showed abundant tetra-nucleated encysted forms of *Entamœba histolytica*. A feature that should be borne in mind in the present case is the symptom of obstinate constipation. Attention is also called to the fact that this occurred in spite of the existence of several minute superficial erosions of the intestinal mucosa as reported at autopsy. Therefore, the criterion, according to the classical definition that "a carrier is one who is clinically normal except that he harbors resistant forms of organisms that may infect other persons," needs revision in the sense that he is not totally devoid of danger to himself.

SUMMARY

1. The case is reported of amœbic abscesses occurring in the brain, lungs, liver, and kidney without concomitant dysentery.

2. The first case of amœbic abscesses of the brain in a Filipino is recorded.

3. A carrier is a source of danger not only to other persons but also to himself.

4. Thorough and repeated fæcal examinations for amœbic cysts should be made in order to discover carriers not only in diarrhœics but also in constipated individuals with complaints of vague pains in the abdomen. Early detection of cases, and intensive treatment with emetine or other amœbicidal substances may save many a patient from a fatal end. Study of the returns of enemata is indicated in a case like this one in which the patient was constipated to a marked degree.

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ILLUSTRATIONS

PLATE 1

Amœbic abscess of the brain. Note the shaggy appearance of the right frontal lobe. A portion of the brain tissue is cut for microscopic study.

PLATE 2

Amœbic abscesses of the liver and lung. The abscess is opened to show the characteristic wall of the abscess. A portion of the lung containing the abscess is seen adherent to the diaphragm.

PLATE 3

Amœbic abscess of the kidney. The abscess is confined to the cortical surface of the kidney.

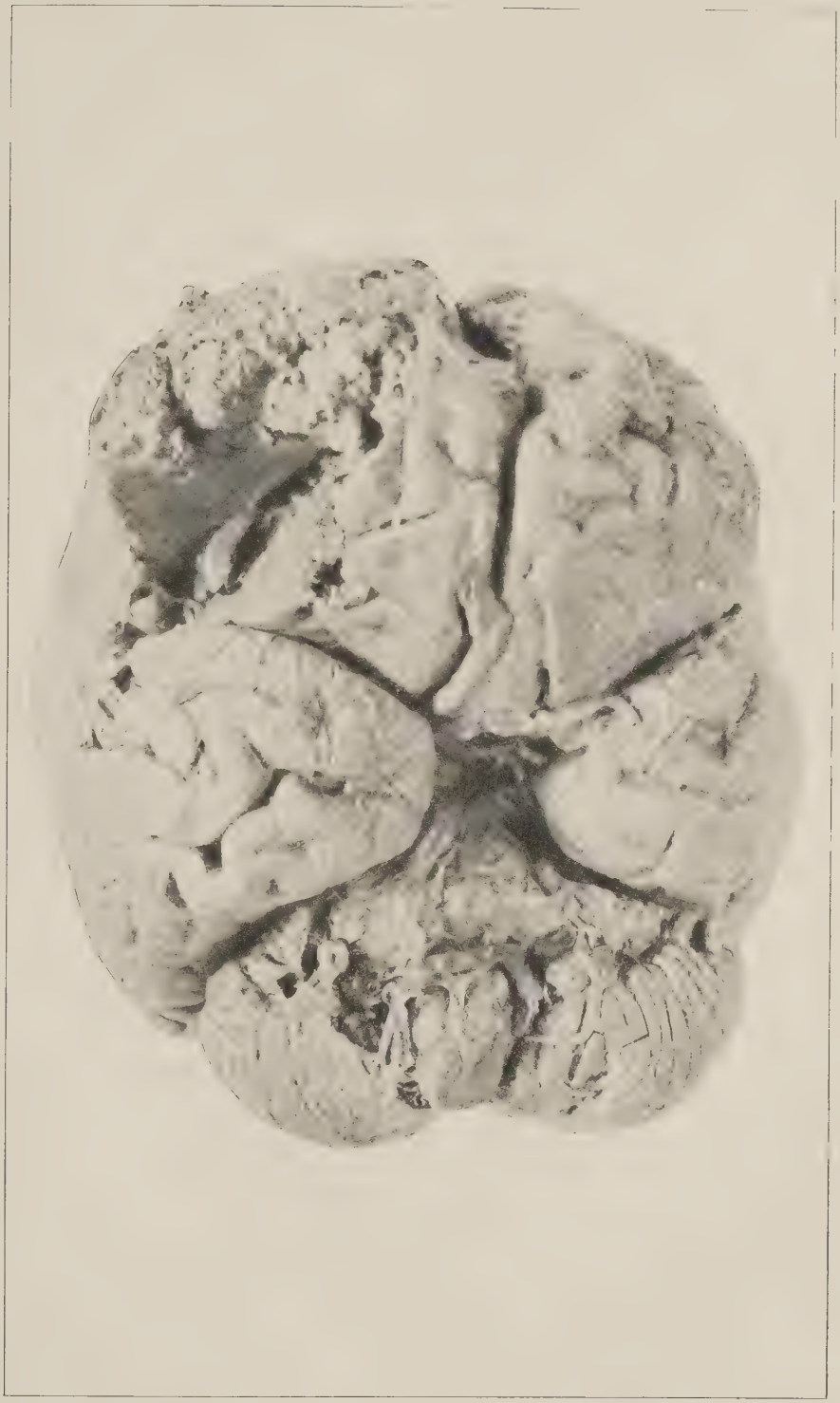

PLATE 1.

PLATE 2.

PLATE 3.

THREE CASES OF POISONOUS INSECT BITE INVOLVING TRIATOMA RUBROFASCIATA

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ONE PLATE

Cases of poisonous bite by insects, centipedes, spiders, and other venomous arthropods are of frequent occurrence in this country. While in many cases the symptoms presented are of such trivial nature that practically no attention is paid to them, in certain instances severe local and constitutional symptoms, more or less alarming or dramatic in nature, are observed. It is only on occasions like these when the interest of the patient or of the family is aroused, that a physician is summoned. When an inquiry is made concerning the offending bug, more often than not the "corpus delecti" cannot be produced, or at most the patient or his family can give only an unsatisfactory description of the animal that inflicted the bite. Consequently the offense is in most cases attributed to almost any kind of bug except the actual offender. It is seldom that the actual offender is caught red-handed.

Recently three cases of severe insect bite with symptoms of actual poisoning were brought to the attention of the writer. In each case the offending bug was captured under circumstances that would permit no doubt as to its culpability. The symptoms observed in the three cases were almost identical, and the captured insects all answer closely the description of *Triatoma rubrofasciata* de Geer, 1773. These three cases are the first of their kind ever recorded in the Philippine Islands, although in many other places this insect has been known to inflict severe bites with marked local and constitutional symptoms. For the interest of Philippine practitioners, case reports and a short description of the insect are given here.

CASE I [1]

I. S. H., male Chinese, 23 years old, single, merchant by occupation, residing in Binondo, Manila, when seen by Dr. Wee was complaining of swollen lips, numbness of the extremities, subcutaneous swellings in different parts of his body, general weakness and pain over the right scapular region which he alleged, was bitten by a bug which he had caught and given to the doctor. According to the patient, he was suddenly awakened by a painful prick over the right scapular region the previous night. Soon after, he felt intense itching at the site of the bite. However, he was able to resume his sleep after some time, but about three hours later he awoke to find himself in severe rigor which lasted for an hour. After the chill he experienced numbness all over his body, which lasted till the next day. Simultaneously his lips became enormously swollen and an enormous wheal appeared on the site of the bite and other parts of the body such as the region around the eyes, nose, and extremities. The doctor was summoned about six hours after the alleged bite. He found the patient lying in bed, apparently weak, with a temperature of 98.2° F., pulse 86, respiration 28, and blood pressure, 120–70. The patient talked with effort due apparently to the swollen lips. The right scapular region was still swollen and inflamed; other similar swellings were found over the eyes, nose, and extremities. No definite mark of the prick could be detected at the alleged site of the bite which was still swollen and œdematous. Pilocarpine was given hypodermically to induce diaphoresis, aspirin to control pain, and strong ammonia water locally to relieve itching. The patient made rapid recovery and in two days all the symptoms disappeared except for the patches of desquamation which marked the site of the swellings.

CASE II

P. P. B., a male Filipino, student, 23 years old, single, residing in Ermita, Manila, came to the University of the Philippines Infirmary complaining of slight headache and reddish swelling of the left upper extremity with hot sensation over the same. He claimed to have been bitten by a bug, which he was able to capture at about 3.30 a. m. of the same day. The site of the prick was at the inferior portion of the left scapular region.

[1] Cases I and II were furnished through the courtesy of Drs. Wee and J. Perez, of Manila, respectively.

The patient felt an itchy sensation all over the left upper and lower extremities just a few minutes after the prick necessitating scratching of the parts. He applied "Katialis" and Sloan's liniment over the itchy portions. He did not notice any wheal formation or swelling of the parts until the time he woke up the following morning, when he found erythematous patches over the left scapular region and diffuse swellings of the left upper extremity. The swellings were very much reddened. He further felt hot sensations over the face, which was also reddened, and over the left lower extremity.

On examination finger scratches were found on the left scapular region. There was noted a solitary erythematous patch about the size of a ten-centavo piece. As in the preceding case, no definite mark of the prick could be seen. The left upper extremity was diffusedly swollen and reddened. The skin over the same was hot. The face and left lower extremity, which he alleged were affected soon after the bite, were found normal. The temperature was 37.5° C.

Complete recovery followed soon after treatment with ammonia water and hot alum acetate compress locally and aspirin and sodium bicarbonate per os.

CASE III

M. S., female Filipino, married, 30 years old, housewife, residing at 193 Fabia, Tondo, Manila, was seen by the writer on the morning of August 20, 1933, complaining of malaise, slight headache, giddiness, tingling sensation in the ears and swelling of the whole right hand and parts of the forearm, which she described as hot, numb, and intensely itchy. She claimed to have been bitten in her sleep on the back of her hand the previous afternoon by a bug which she presented to the writer. The site of the prick was so itchy that she had to scratch it furiously. Soon after the back of her hand became numb and began to swell, the swelling rapidly extending to contiguous parts, so much so that by nightfall of the same day all her fingers and wrist were involved. That night she was not able to sleep well on account of the intense itching of the affected parts. On examination about twenty hours after the bite, the patient was found feverish with slightly accelerated pulse. The whole right hand, including the lower half of the forearm, was diffusedly swollen, hot, and reddened. The fingers of the affected hand were blotched and flexion was difficult (Plate 1, fig. 1). At the base of the middle finger, on the back of the

hand, could be detected a small weeping puncture about half a millimeter in diameter, presumably the site of the prick. No other swelling was found in other parts of the body. Pyramidon internally and strong ammonia water locally were given and the patient was allowed to go home. When the patient returned two days after, no trace of the swelling could be found except a patch of desquamation at the site of the prick.

Triatoma rubrofasciata is a blood-sucking reduviid which is known, wherever it is found, to attack man and other vertebrates inflicting on its victim a severe bite, which is at times followed by severe general symptoms, such as swelling in different parts of the body, nausea, etc., as well as much local pain and irritation.

The character of the symptoms observed in cases of *Triatoma* bites suggests both inflammatory and neurotoxic poisons. The nature of the venom is still unknown but probably it is a mixture of highly complex proteids similar to the venom of bees and wasps. Cornwall and Patton(4) found in it a highly developed anticoagulin. The severity of either local or general symptoms would depend on whether the poison is injected subcutaneously or into a vein. If the poison is introduced subcutaneously, the general symptoms are much less pronounced than the local manifestations as it would take longer for the venom to be absorbed by the blood and distributed to the different parts of the body. On the other hand, the effect of entry into a blood vessel is to produce immediately constitutional symptoms, which are as a rule more alarming than the local ones. The degree of poisoning would also depend to a large extent on whether or not the insect has recently spent its stored venom on other victims. These different factors tend to explain the varying severity of bites alleged to be inflicted by this insect. Thus Patton (1913), who has fed a large number of adults and nymphs of this insect on himself and has recorded the effects of their bites, failed to mention any constitutional symptoms. Among other things he mentioned that twelve hours after the bite a large erythematous patch about the size of a shilling appeared at the site of the puncture; it remained in an inflamed and extremely irritable condition for about a fortnight and then faded away; a small nodule about the size of a No. 6 shot persisted for about a month.

The appearance of local œdematous swellings in the different parts of the body of the victim suggests a kind of toxin that

upsets the vasomotor mechanism in certain susceptible areas of the body. The importance of this phenomenon may readily be appreciated by bearing in mind the possibility of the glottis becoming the site of such vasomotor disturbances and consequent œdema.

It is interesting to note that in two of the foregoing case reports, the site of the bite was in the scapular region, and that the prick in both cases occurred while the patient was presumably in the recumbent position. This seems to support the contention of Patton and Cragg (1913) that this insect attacks man only under exceptional circumstances, as when it is carelessly handled. The senior author was able to feed as mentioned above, a large number of adults and nymphs of this insect on himself. The authors did not, however, mention whether or not these adults and nymphs were carelessly handled.

The insect is large, measuring from 19 to 24 millimeters in length, and is easily recognizable. In general appearance it resembles the common garden bug, or "vaca-vacahan," but it is much larger and differently colored. The eyes are very prominent and form two lateral rounded swellings at the sides of a long roughly rectangular head; the ocelli, which are behind and above the eyes, are well developed, and often appear as clear spots. The proboscis is short and stout and is held in a looped manner under the head (Plate 1, fig. 2). The following is the description of this insect by Patton and Cragg (1913).

Generic Diagnosis

GENUS TRIATOMA WOLF, 1802

Reduviidæ. Head long, porrect, more or less distinctly impressed behind the eyes; rostrum with the first joint very much shorter than the second; antennæ inserted on the sides of the head about midway between eyes and apex; ocelli placed very far apart; prosternum broadly sulcated; abdomen strongly ampliated, not centrally carinate, frequently with the disk prominently flattened; posterior tibiæ longer than the femora. (Distant.)

Specific Diagnosis

TRIATOMA RUBRUFASCIATA DE GEER, 1773

Pisceous brown. Head dark brown to black with the basal margin reddish. Antennæ with first joint not quite reaching apex of the head; second joint three times the length of the first; basal joints dark, apical joints light. Pronotum dark brown to black; anterior angles produced into two short spines of a reddish yellow color. Two blunt prominences ending in diverging ridges on anterior portion of dorsal surface of pronotum; lateral margin of pronotum to posterior angles with a reddish yellow linear

streak; scutellum dark, apex occasionally reddish; corium with a basal oblique linear streak and a somewhat diffuse subapical reddish yellow spot; wing membranes fuscous. Connexivum with reddish yellow lateral spots on dorsal surface, which as a rule extend to the margin and form a continuous reddish yellow edging to the abdomen. Length 19 to 23 mm. (Patton and Cragg.)

Triatoma rubrofasciata has a very wide geographical distribution, although it is believed to be a true Oriental species which has spread to other areas. It has been reported in Brazil, Haiti, Argentina, China, Madagascar, Sierra Leone, Mauritius, Zanzibar, Tanga, Sumatra, New Guinea, Indo-China, Borneo, Java, Ceylon, Singapore, Formosa, Japan, and the Philippines. In the entomological collection of the Department of Parasitology, School of Hygiene and Public Health, University of the Philippines, the author found four specimens labeled *Megistus rubrofasciatus* de Geer (= *Triatoma rubrofasciatus*) by Banks in 1915. They were collected in Manila and vicinity. Specimens of this species were also collected by the author in the same localities. The adult seems to frequent vines like squash, "opo," "patola," and the like. Patton and Cragg (1913) were not able to encounter the early stages of this insect in holes in the ground or about roots of trees after intensive search in localities where adults are common, indicating that this insect is arboreal in habit. According to Comstock (1910) nearly all reduviids live upon trees and other plants and prey upon other insects. This insect frequently enters houses at night, and if the alimentary tract of the insect is dissected out, it will be found to contain mammalian blood. Whether or not it feeds regularly on human blood is not known. If it does, it is worth bearing in mind that *Triatoma megista*, a closely allied species, is the confirmed invertebrate vector of Chagas fever, a fatal form of human trypanosomiasis in many parts of Brazil. Pathogenic trypanosomes, as shown by the behavior of the causative organisms of sleeping sickness, seem to show a tendency to use more than one invertebrate intermediary host. For this reason, *T. rubrofasciata* as a potential vector of Chagas fever, should be considered. The possibility of introducing this dangerous disease to new areas does not seem improbable, especially if we consider the fact that it runs a chronic course and does not prevent its victim from making long trips to other places or from settling in new areas. Because of modern facilities of transportation, the world's population is becoming more and more mobile, and diseases which were formerly more or less

confined within limited areas have been found to have crossed mountain and ocean barriers with the stream of human migration. Thus we see that *Diphyllobothrium latum* infestation has gained a permanent foothold in the region around the Great Lakes of America while *Schistosoma mansoni* infection has become endemic in the West Indies and parts of South America. The former was introduced into the new endemic area presumably by the Fins and Scandinavians who migrated from the region around the Baltic Sea where this tapeworm is a common infection, and the latter by the Negro slaves who were brought to the New World from Africa in the early part of the seventeenth century. Since these two worms cannot establish themselves in areas where their respective intermediary hosts are absent, it is evident that their vectors or at least species closely allied to them, are present in the regions where they have been recently introduced and remained endemic. As a large number of Japanese are now populating different parts of Brazil, it does not seem far fetched to conceive of a similar situation arising in the future whereby Japanese immigrants visiting their home country and other areas in the Far East, may introduce Chagas fever into these virgin areas by virtue of the presence of *Triatoma rubrofasciata,* a cogeneric species of the confirmed vector of this disease in Brazil. Recently Kofoid and Donat (1932) succeeded in transferring experimentally a trypanosome which they believed (as a result of a long and painstaking investigation) to be identical with *Trypanosoma cruzi,* from experimentally infected *Triatoma protracta* to mammals in California.

It might be mentioned also that *Triatoma rubrofasciata* is commonly infected with a flagellate, *Crithidia conorhini* Donovan, in Madras where it has been suspected, though on slender evidence, as a transmitter of kala azar. In Mauritius Lafont has found 80 per cent of this insect infected with a similar flagellate with which he was reported to have succeeded in infecting rats and mice with a trypanosome by inoculating it intraperitoneally. As most of his animals died apparently on account of the infection, he draws attention to the possible rôle this flagellate may play in the case of man. Another line of investigation, suggested by the presence of this insect in this country, is the possible rôle it may play in the transmission of surra, another trypanosome disease which causes a large number of deaths among horses every year. The fact that this insect seems to be arboreal in habit, living as it does among domestic

vines and other plants and in close proximity to the common haunts of our domestic animals, and the further fact that it has been found to feed avidly on mammalian blood in nature and in captivity, would strengthen suspicion in this direction.

SUMMARY

Three authentic cases of *Triatoma* poisoning, believed to be the first recorded in the Philippine Islands, with local and constitutional manifestations usually observed in bites by this insect as reported in other countries, are discussed in this paper. The possible rôle of *Triatoma rubrofasciata* in the transmission of important blood-flagellate infection is discussed.

ACKNOWLEDGMENT

The writer is gratefully indebted to Dr. Jose Perez and Dr. Wee, of Manila, for their courtesy in placing their clinical data at his disposal.

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ILLUSTRATION

PLATE 1

FIG. 1. Case 3, right forearm and hand, showing the swelling caused by *Triatoma* bite.

2. *Triatoma rubrofasciata* de Geer; female.

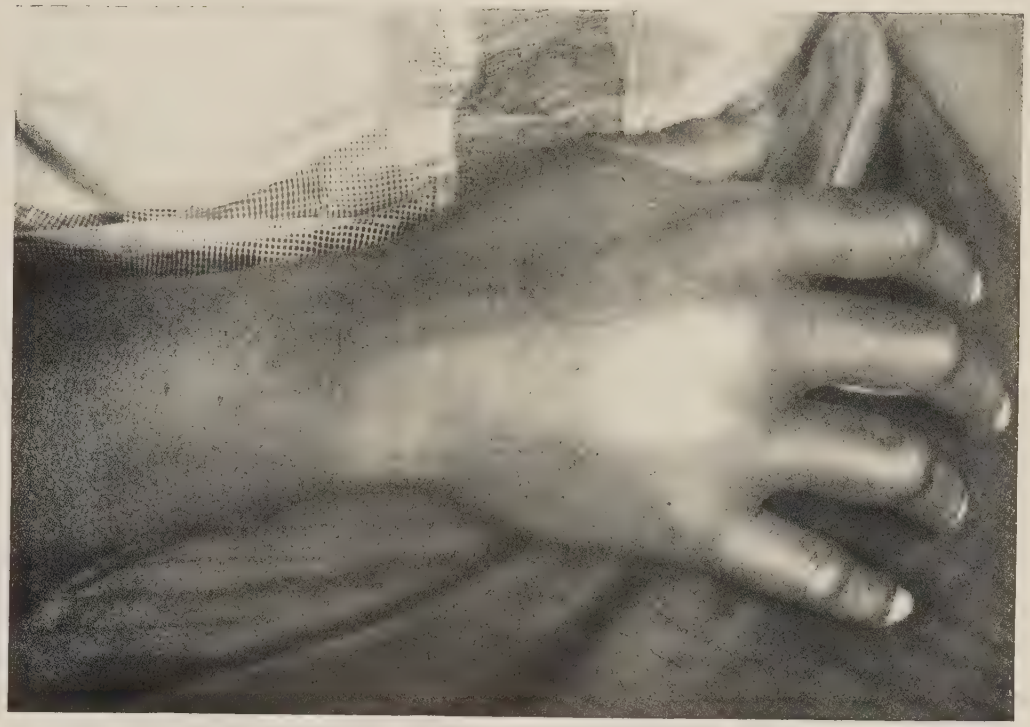

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PLATE 1.

EXPERIMENTAL INQUIRY INTO THE TRANSMISSION OF RAT-BITE FEVER AMONG RATS, PART II

SUCCESSFUL TRANSMISSION OF RAT-BITE FEVER BY FEEDING INFECTED ORGANS TO WHITE RATS

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In part I[2] of this investigation I summarized the results of my experiments concerning this subject. Attempts to transmit rat-bite fever to white mice by offering them guinea pig's organs containing *Spirochæta morsus muris* as food, failed to convey the infection to these experimental animals. It was observed, however, that white mice do not take raw meat and organs readily as food and for this reason further experiments were arranged in which laboratory white rats were used instead of white mice. Under such an arrangement the experiments were successful, for white rats, like their wild gray cousins, devour raw organs readily. Furthermore, the fact that white rats are more closely related to wild rats than are white mice, gives added weight to the present experiments.

METHODS

The methods of feeding infected material to the experimental rats were as follows:

1. Organs and tissues, such as blood, liver, spleen, kidneys, and suprarenals, of guinea pigs infected with Manila strains of *Spirochæta morsus muris* were given to white rats to eat.

2. Blood withdrawn from infected guinea pigs by heart puncture was mixed immediately with cow's milk and given to the rats as food.

In the first instance the infected donor, as can be surmised, had been sacrificed and in the second method the blood was withdrawn from a living infected guinea pig. In either case the presence of spirochætes in the material offered as pabulum

[1] Surgeon Lieutenant Commander, Imperial Japanese Navy.

[2] Philip. Journ. Sci. 52 (1933) 89–95.

was ascertained by dark-field microscopic examination immediately before feeding. The pabulum prepared in one way or the other was readily taken by the white rats.

In judging the results of the experiments, we have not satisfied ourselves by merely noting the symptoms of the infection, but considered a transmission experiment as successful only when spirochætes were demonstrated by microscopic examination either directly in the blood of infected rats or in that of guinea pigs to which blood or the organs of rats experimented on have been transferred by inoculation.

EXPERIMENTS

Three normal, laboratory-bred white rats (R–1, R–2, and R–3) were used in one set of experiments. To facilitate infection I have artificially scarified the gums of one of the three animals (R–1). The other two were left without interference.

To these three animals infected guinea pig's organs were fed December 20, 1932, and January 10, 1933. They ingested infected blood January 4, 5, 17, and 20, 1933. During these dates they were also fed freely on milk mixed with blood containing spirochætes.

The blood of the white rats was examined daily beginning December 20, 1932, either fresh under the dark-field microscope or in the form of stained blood smears, but spirochætes were not found. It appeared, therefore, that the results of this experiment, like those on white mice, would be negative. The possibility, however, occurred to us that the rats may be relatively immune and, consequently the spirochætes invade the blood stream, if at all, in such small numbers that even careful and repeated examinations fail to reveal them. In order to decide this point I infected one of the rats (R–2) with material rich in spirochætes by subcutaneous injection and examined the rat's blood at frequent intervals of time. As I suspected that the spirochætes, if found at all, would be very few and hard to find, I began to take the blood with distilled water in order to eliminate the interference of red blood cells in the search for spirochætes. On the forty-second day after infection one typical spirochæte was found by dark-field illumination, but the search in stained smears was fruitless. In order to confirm this finding I withdrew blood from the same rat and injected it into a guinea pig, which in due time developed the disease with positive finding of spirochætes. Thus it was learned that under the most

favorable conditions *Spirochæta morsus muris* rarely invades the blood stream of white rats and in small numbers at that.

A similar procedure, however, performed on rat R-1 January 11, 1933, failed to produce the disease in a guinea pig inoculated with the rat's blood. This rat, it will be noted, ingested repeatedly infected organs, blood, and milk, but has never been injected with spirochætes.

In view of these difficulties, I sacrificed the two remaining rats that had been fed repeatedly on material rich in *Spirochæta morsus muris* and separately inoculated normal guinea pigs with the organs of these rats. The guinea pigs promptly developed rat-bite fever with positive findings of the specific parasites. To check the results two other white rats (R–4 and R–5) were fed with the organs of these animals February 27, March 30, and April 3, 1933. These rats were killed April 14, 1933, and their organs were inoculated separately to two clean guinea pigs. One of these guinea pigs developed rat-bite fever, while the other failed to show any signs of the infection during a period of observation that lasted forty-six days.

From the results of the above experiments, we believe the following conclusions are justified:

CONCLUSIONS

1. White rats, more than white mice, are partially resistant to Manila strains of *Spirochæta morsus muris* that have been proven to be very virulent for guinea pigs, rabbits, and Philippine monkeys.

2. Experimental evidence presented in this communication points to the possibility of transmission of rat-bite fever among rats by the ingestion of recently dead infected rats.

ACKNOWLEDGMENT

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THE PHYSIOLOGY OF REPRODUCTION IN SWINE, I [1]

THE SEMEN OF BOARS UNDER DIFFERENT INTENSIVENESSES OF MATING

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ONE TEXT FIGURE

The study of the physiology of reproduction in swine is of great theoretical and practical interest; and the Soviets, under whom these studies were begun, admit that the importance of the theoretical aspect of the work is enhanced by the fact that it sheds light upon the practical or utilitarian aspects. It is hoped that ultimately such studies may lead to a better theoretic understanding of the reproductive processes, and as a result bring about the possibility of devising more rational breeding practices. These studies, however, hardly scratch the surface, and the writer cannot attempt any more than to point to some of the more salient biological problems involved.

EXPERIMENTAL WORK

Object of the experiment.—This experiment was planned primarily to determine the quantity of sperm that is ejected by a boar under different intensivenesses of mating. First, under a light mating schedule, the volume of the semen and the density and the total number of spermatozoa in the whole ejection were determined, and after that the effect upon these quantities of different intensivenesses of mating. In the course of the experiment, we noted the mating behavior of the boar, the activity of the spermatozoa in the semen from the first mating of the boar, and also certain morphological characteristics of the spermatozoa.

[1] The data presented in the first three papers of this series were worked out in the "All-Union Research Institute for Swine Husbandry," Poltava, Ukraine, U. S. S. R., with which the writer was connected as geneticist during the period 1930–1932.

Material.—At the beginning of the experiment there were twenty-four Yorkshire boars. These were taken from stock which had been in the Ukraine a number of generations, and were apparently fully acclimated. Two were over 4 years old, one was 3 years old, two were 2 years old, and the rest were 1 year old or less. There were six 8-month-old boars. The two oldest boars were to be used in determining the effect of age, if any, upon the production of sperm, but they would not mate properly.

Ration.—The ration of the boars consisted of a grain mixture containing barley, 75 parts; bran, 15 parts; sunflower oil meal, 10 parts. The amount given each animal depended upon his weight, as required by the unit system of feeding. Because of difficulties connected with the general grain shortage in the country, this mixture was somewhat altered temporarily three times during the course of the work. When such an alteration was made, collection of semen was suspended for at least two days thereafter.

A simple mineral mixture consisting of 20 parts common salt (NaCl) and 80 parts calcium carbonate ($CaCO_3$) was also given.

The ration was supplemented by greens eaten by the animals in the course of their daily exercise in the grassy yard.

General care.—All the boars were housed in the same barn. They were given at least an hour's walk every day. The pens were washed daily, and the animals themselves were washed and scrubbed once a day.

METHODS

Method of collecting the semen.—An old method of collecting the semen of domestic animals consisted in inserting into the vagina a piece of sponge which absorbed the semen, and then withdrawing it to squeeze out its contents. Experience showed that this method cannot be used with the sow. First, because of the narrowness of the vagina of the sow, and, just as important, because of the presence of the sphincter between the vestibule and the vagina, it is impossible to insert a sponge large enough to absorb all the boar's semen. We know now that the boar secretes an average amount of 252.8 cc of semen, a volume that could be absorbed only by a sponge too large to insert into the vagina. Second, the secretion by the boar of the vaginal plug would probably interfere even if we could insert a sponge of adequate size. Third, as a sponge has no selective power of ab-

sorption, it would absorb not only the semen but also any other secretions in the vagina. Fourth, a sponge has to be squeezed, and not all the semen could be squeezed out. A considerable quantity would remain adsorbed by the sponge.

The foregoing considerations show that a mechanical means of collecting the semen is a desideratum, for it would make possible the collection of all the semen ejected in pure condition. McKenzie (1931) first devised a method of mechanically collecting semen from the boar. This method consists in having the penis inserted into a soft rubber tubing which is so manipulated as to impart to the penis a pulsating action. It is doubtful, however, if the full ejection was obtained by the use of this method. A more convenient instrument, the artificial vagina, was later invented in the Laboratory of Artificial Insemination in Moscow, and somewhat improved in the writer's hands (fig. 1).

The artificial vagina is very simple to construct and to prepare for use. The bicycle inner tubing must be put on by folding its ends over the ends of the larger tube, the sides of which are insulated in order that the temperature of the water may be kept fairly constant for the required period of time. That done, warm water (temperature, 40 to 42° C.) is introduced into the cavity between the bicycle inner tubing and the larger tube through the side tube *b*, with the stopcock on the side tube *a* open to allow the air that is displaced by the water to escape. Enough warm water should be put in so that as much of the air as possible is displaced. Then side tube *a* is closed by means of the stopcock. The rubber bulb is attached tightly to the side tube *b*.

The funnel-shaped piece of rubber at the opening of the vagina acts as a guide for the penis to get into the slitlike canal of the artificial vagina. At the opposite end is the glass vessel for collecting the semen. Once the penis gets into the artificial vagina, pulsation is produced by exerting and releasing pressure on the rubber bulb.

The pulsating action is necessary for the completion of the coitus, in the case of animals which take a long time to complete the sexual act, but for those which complete the coitus simultaneously with the insertion of the penis, as in the case of the ram, it is not necessary.

At first the lumen of the artificial vagina was smeared with vaseline the action of which on the spermatozoa had previously been tested. Experience has shown, however, that it is not at

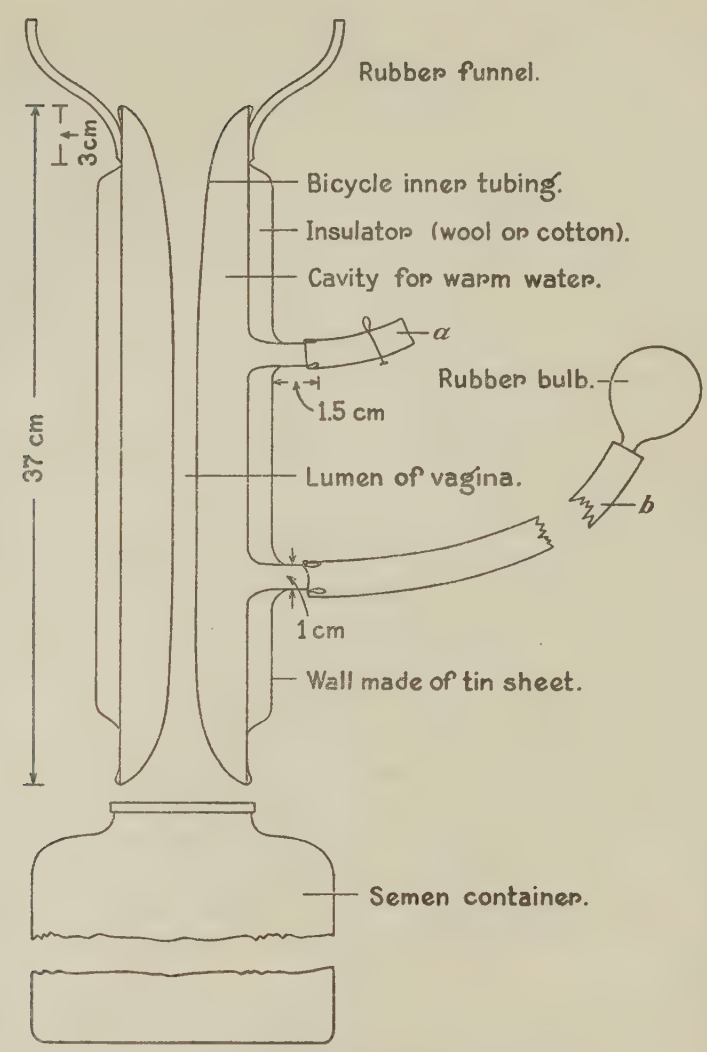

FIG. 1. Longitudinal section of artificial vagina.

all necessary to use vaseline, for when the glans penis meets with the opening of the vagina the first two secretions are squirted, and these secretions are a sufficient lubricant for the penis. There is the advantage also of having no foreign substance incorporated in the semen.

The boar is allowed to mount a sow, and the penis is taken and directed into the artificial vagina, which is held by the other hand. McKenzie noted that the sow does not have to be in heat; she can be tied by the upper jaw to a post, and to prevent her from side-stepping the use of the breeding crate is very convenient. Later, a dummy sow was found to be much more

convenient to use as a decoy, for it does not have to be tied and it does not side-step. Besides, the vagina may be placed as in the natural sow, so that one does not have to bother holding it. The use of the dummy is also practicable for the stallion and the bull. I have seen a wether used instead of a ewe as a decoy.

The matings.—A calendar of mating was made out and followed as closely as conditions permitted.

Measurement of the volume of the semen.—The volume of the semen is measured by means of an ordinary graduated cylinder. This is accurate within 5 cc. The ease with which foam is formed and the relatively large diameter of the cylinder are the principal sources of error. For a semen sample of 300 cc or more this is a small error; for smaller samples, it is proportionately greater.

Counting the spermatozoa.—The number of spermatozoa per cubic millimeter was determined by means of the Thoma-Zeiss blood counter. We took 1:10, 1:20, 1:50, or 1:100 dilutions of the semen, depending upon the density of the spermatozoa. Toisson's fluid was used as diluent. The counting was done as in making the familiar blood counts. At least two counts of each sample were made, and their average was taken as the correct value.

Records.—The date of each mating, the volume of the semen, the density of the spermatozoa (that is, the number of spermatozoa per cubic millimeter), and the total number of spermatozoa in the ejection were recorded.

Preparations.—At least three slides were prepared from each sample of semen. The fixation was done by heat, followed, after cooling, with immersion in 95 per cent alcohol. The slides were then stepped down through the different grades of alcohol to the aqueous stain. Staining was done with Heidenhain's iron-hæmatoxylin. The hæmatoxylin was ripened by Shortt's method.

THE SEMEN

The semen from the first mating of the boar.—There is a current belief that the spermatozoa in the semen from the boar's first mating in life and from the first mating in a breeding season are dead or in some way nonfunctional. For a few cases this idea apparently holds true. Of the nineteen boars from which we were able to gather semen during the course of the experiment four were 2- to 3-year-old boars, three of which

had already mated before, and one, boar 1883/284, never had mated; the rest were young boars that were being mated for the first time. These, therefore, constituted good material for testing this belief.

Bogdan /61, a 13-month-old boar mating for the first time, mated normally, but during the early part of the experiment gave only nonmotile spermatozoa. His record is given in Table 1.

TABLE 1.—*Semen from boar Bogdan /61.*

Date of mating.	Volume of semen.	Condition of spermatozoa.
	cc.	
May 25	175	Nonmotile.
May 29	60	Do.
June 2	65	Do.
June 7	105	Do.
June 13	75	Do.
June 15	96	About 1 per cent of the spermatozoa were motile.
June 19	160	About 95 per cent of the spermatozoa were motile.
July 2	127	Almost all were motile.
July 5	225	Do.

Two other boars gave nonmotile spermatozoa, though their record was far from being as bad as that of boar /61

TABLE 2.—*Semen from boars 2200/91 and 2703/190.*

Boar No.	Date of mating.	Volume of semen.	Condition of spermatozoa.
		cc.	
2200/91	June 1	510	Nonmotile.
2200/91	June 14	200	Do.
2200/91	June 16	315	About 90 per cent were motile.
2703/190	June 11	35	Nonmotile.
2703/190	June 27	312	About 60 per cent were motile.

Boar 2200/91, a 2-year-old animal, had mated before, while 2703/190, a 12-month-old boar, was mating for the first time.

Since the summer of 1931 we had an opportunity to mate most of the nineteen boars once more after five months of sexual inactivity. Boar /61 was remated December 13, 1931. He gave 215 cc of semen in which 50 per cent of the spermatozoa were nonmotile and agglutinated. January 4, 1932, he gave 350 cc of semen containing spermatozoa that were all nonmotile.

January 7 he gave 309 cc of semen with nonmotile spermatozoa; January 10 he gave 290 cc with almost 100 per cent motile spermatozoa. Boar 2703/190 was remated January 9, 1932. He gave a sample of semen in which the spermatozoa were practically all nonmotile; only about 0.5 per cent were active. From subsequent matings, however, only motile spermatozoa were obtained. Boar 2200/91, on the other hand, gave motile spermatozoa January 10 and on two subsequent matings.

These three cases were interesting because they form exceptions to the rule. The sixteen others gave very lively spermatozoa on the first and subsequent matings. Since the former were given the same care and food, and kept under the same conditions as the sixteen others, the phenomenon of the presence of dead spermatozoa in their semen can hardly be attributed to environmental conditions. For, if these were responsible, the former should have continued to give nonmotile spermatozoa, for one thing, and for another, the other sixteen boars should have produced semen with nonmotile spermatozoa, which they did not. One would explain this condition as a case of the presence of gametic lethal genes were it not for the fact that after a few matings active spermatozoa were again obtained.

The real explanation is probably to be found in some phenomenon or phenomena of a physiological nature. The writer's hypothesis is that these three abnormal boars were cases of auto-immunization. It is assumed that they, like other boars, produced spermatozoa continually; but, unlike the normal ones, they somehow resorbed their spermatozoa as these accumulated in the seminal vesicles or in other sexual organs, as in the testes themselves or in the epididymis. It is further assumed that as resorption took place, a parallel production of antibodies against the spermatozoa occurred. These antibodies, by continually acting upon the spermatozoa, eventually killed them. This, it may be supposed, happened when boars /61, 2703/190, and 2200/91 were not mated for a long time. Later, when they were mated often—that is, as the interval between matings was shortened—two processes took place: (*a*) less spermatozoa were resorbed and the production of antibodies diminished; and (*b*) the time during which the antibodies acted upon the spermatozoa was shortened, and their lethal action lessened.

The plausibility of this explanation is supported to some extent by Guyer (1922, 1925), who reported success in producing

sterility in rabbits, which previously had showed fertility, by injecting into them their own spermatozoa. Furthermore, by showing that if the lens of the eye is injured the animal produces antibodies against its own lens, Guyer gave a specific foundation for the idea that an animal, the rabbit, at least—under certain conditions, does produce antibodies against its own tissues. By analogy, it may be supposed that the boar upon resorption of his own spermatozoa develops auto-immunization. This would account for the nonmotile and agglutinated condition of the spermatozoa of the three boars above mentioned.

The semen of boars under a light mating schedule.—The following table is a summary of the data on boars under a light mating schedule:

TABLE 3.—*The age of the boars, the volume of the semen, and the density and total number of spermatozoa given during a light mating schedule.*

Boar No.	Age.	Mated.	Volume of semen.	Spermatozoa.	
				In 1 cmm.	Total.
	Mos.		*cc.*		$\times 10^{10}$
2782/298	10	June 28	402	198,000	7.96
		June 30	217	502,000	10.89
		July 29	135	587,500	7.93
2784/302	10	July 15	135	233,000	3.15
		Aug. 11	105	372,000	3.91
		Aug. 23	230	281,000	6.46
2805/330	10	July 11	180	178,000	3.20
		July 22	160	258,000	4.13
2807/334	10	June 28	175	143,000	2.51
		June 30	335	61,200	2.05
		Aug. 13	200	67,000	1.34
2688/170	12	June 29	295	181,000	5.34
		Aug. 4	180	311,000	5.60
		Aug. 7	170	344,000	5.85
2695/178	12	May 31	250	595,000	14.87
		June 5	160	830,000	13.28
		July 30	230	464,000	10.67
2705/194	12	May 31	200	720,000	14.00
		June 5	240	486,000	11.66
		June 8	340	312,000	10.61
2707/196	12	June 11	240	376,000	9.02
		Aug. 12	180	570,000	10.26
		Aug. 22	380	430,000	16.34
2709/198	12	June 27	290	194,000	5.63
		June 29	120	598,000	7.18
		Aug. 2	250	184,000	4.60
2564/34	14	June 10	315	418,000	13.17
		June 22	140	1,004,000	14.06
		July 26	260	419,000	10.89

TABLE 3.—*The age of the boars, the volume of the semen, and the density and total number of spermatozoa given during a light mating schedule*—Continued.

Boar No.	Age.	Mated.	Volume of semen.	Spermatozoa.	
				In 1 cmm.	Total.
	Mos.		*cc.*		$\times 10^{10}$
2485/27	14	May 25	220	578,000	12.72
		May 29	350	294,000	10.29
		June 2	410	286,000	11.73
2486/28	14	May 25	260	422,000	10.97
		June 13	235	193,000	4.54
		July 18	295	239,000	7.05
/61	13	May 25	175	372,000	6.51
		July 2	127	227,000	2.88
		July 13	245	325,000	7.96
2615/91	13	June 9	315	338,000	10.65
		June 21	340	364,000	12.38
		June 24	265	244,000	6.47
2196/87	24	June 1	410	31,400	1.29
		July 16	155	169,000	2.62
		Aug. 1	190	276,000	5.24
2200/91	24	June 1	510	23,000	1.17
		July 3	420	92,000	3.86
		Aug. 1	310	263,000	8.15
1883/284	34	May 28	190	480,000	9.12
		July 3	249	400,000	9.96
		July 17	540	253,000	13.66
Average			252.8	339,354.9	7.83

DISCUSSION OF DATA PRESENTED IN TABLE 3

1. There is no correlation between the volume of the semen and the total number of spermatozoa. A boar will sometimes give more spermatozoa in a small volume of semen than in a large amount. The case of boar 2200/91 is a conspicuous example. June 1 this boar gave 510 cc of semen, which contained only 1.17×10^{10} spermatozoa. August 1 he gave a much smaller amount of semen, 310 cc, but the total number of spermatozoa was much greater, 8.15×10^{10}. The volume of the latter was three-fifths that of the former, though the latter sample contained 7.4 times as many spermatozoa as the former. In the former case the greater volume of liquid acted as a mere diluent. There are other similar cases. Boar 2709/198 June 29 gave 120 cc of semen containing 7.18×10^{10} spermatozoa; August 2, 250 cc containing only 4.60×10^{10}. The table shows still other cases.

On the other hand, there are cases where a greater volume of semen means a greater number of spermatozoa. There is the case of boar 2707/196, to mention but one, that June 11 gave 240 cc of semen containing 9.02×10^{10} spermatozoa; and August 22, 380 cc containing 16.34×10^{10} spermatozoa.

The lack of correlation between the volume of semen and the number of spermatozoa carried in it compels one to consider both elements in examining the sexual potency of the boar.

2. The volume of the semen varies a great deal even in the same boar; under a light mating schedule it may range from 190 cc to 540 cc, as in the case of boar 2200/91. In Table 3 the volume of the semen varies from 105 cc to 540 cc; the arithmetic average is 252.8 cc.

3. The number of spermatozoa ejected by a boar, like the volume of his semen, varies widely. In this respect boar 2200/91 is a good example. June 1 he gave only 1.17×10^{10} spermatozoa; July 3, 3.86×10^{10}; and August 1, 8.15×10^{10}. Other boars also showed a great deal of variation, though not as much as that shown by boar 2200/91. The table as a whole shows a range of variation from 1.17×10^{10} to 16.34×10^{10}, the arithmetic average being 7.83×10^{10}.

The number of spermatozoa contained in the semen is very important, because the number of functional spermatozoa that a boar gives in one ejection is a measure of his fecundity—fecundity being defined as the ability of producing functional gametes. This definition of fecundity suffers from the indefiniteness of the term "functional." For present purposes, until the different factors constituting what must be meant by this term "functional" have been studied, it must be assumed that a functional spermatozoa is one that is actively moving forward in the normal rotatory manner. From a practical standpoint, the knowledge of the fecundity of a boar is important because a boar that produces relatively few functional spermatozoa (a boar of poor fecundity) is more likely to have poor fertility, and therefore may be safely discarded. Knowing the fecundity of a boar one can foretell his probable usefulness as a breeder.

It is assumed that there is a fairly close correlation between fecundity and fertility. This assumption is based on what is known to happen to the spermatozoa in the uterus after insemination, at least in the case of some laboratory animals. Yochem (1929) presents data to show that the number of spermatozoa in the genital tract of the guinea pig is reduced greatly

in the course of twenty-four hours, and that the leucocytes seem to be largely responsible for this. It is, of course, a common fact that during estrum the uteri of all animals so far studied contain in their lumen great numbers of leucocytes. Although the writer's evidence is not as yet conclusive, he has made some observations which tend to show that this phenomenon of the spermatozoa being consumed by the leucocytes takes place also in the sow's uterus. These side lights make it more possible to understand clearly the rôle played by the great numbers of spermatozoa that a boar gives in one ejection: the greater the number of spermatozoa the greater the probability that enough spermatozoa will survive the action of the leucocytes to fertilize the ova. Herein, therefore, is one factor that acts to correlate fecundity and fertility.

4. The density of the spermatozoa varies a great deal, even in the same individual. To take two well-marked examples: Boar 2196/87 showed a density of 31,400 spermatozoa per cubic millimeter June 1, 169,000 July 16, and 276,000 August 1. Boar 2200/91 gave a similar record: June 1 he gave 23,000 spermatozoa per cubic millimeter, July 3, 92,000, and August 1, 263,000. Taken as a whole Table 3 shows that the variation ranges from 23,000 to 1,004,000 spermatozoa per cubic millimeter. The arithmetic average is 339,354.9 spermatozoa per cubic millimeter.

The density of spermatozoa in the semen is very important. Unless the volume of the semen is very large, small density means a small total number, and this means, from the above definition, poor fecundity. There are at least five factors to which poor density may be attributed; namely, (*a*) failure of the boar to mate normally, which may mean the secretion of a large amount of the first two secretions, (*b*) some physiological abnormality of the boar, (*c*) sexual immaturity, (*d*) senility, (*e*) poor fecundity. The first four may easily be ruled out.

5. The age of the boar is of course a factor in the production of semen. As stated above, of the six 10-month-old boars that we had in the experiment, we succeeded in getting only four to mate. From the standpoint of both the average of the density and the total number of spermatozoa, boar 2807/334 gave poor samples of semen, and so did two other young boars. Boar 2782/298, on the other hand, by giving good samples of semen (from the above standpoint) was clearly an exception among the young animals.

As to the average of the density and of the total number of spermatozoa, it was found that the 12- and 14-month-old boars almost always gave good semen. Not all mature boars, however, produced good semen. Despite the fact that his semen was more voluminous than the average sample, boar 2200/91 gave such a low density of spermatozoa that the total number was far below the average. This was true in two of the three samples cited. In the third case the boar gave a density and total number of spermatozoa that was higher than the average. Boar 2196/87 failed to give a sample of good semen in the three cases mentioned in the table. A 12-month-old boar, 2688/170, like the last two, failed also to give semen that contained the average total number of spermatozoa.

It appears from the above table that, as a general rule, 10-month-old boars fail to produce as much spermatozoa as mature boars—1.5-year-olds, for instance—though the number of young boars studied was small, and the data are too meager to warrant dogmatic statements about the use of young boars as breeding animals. From the available data one may merely infer that it is probably not wise to breed 10-month-old boars. Not only did some of the young boars fail entirely to mate when tried, but the ones that did mate, except one, failed to produce the average total number of spermatozoa. We must also bear in mind the common knowledge that breeding a young animal tends to retard its growth.

On the effect of old age upon the production of spermatozoa no data were obtained. A study of this problem not only would shed light upon the sexual life of the boar, but also determine how long and to what age the boar may be kept profitably for breeding purposes.

The semen in intensive mating.—By intensive mating is meant, in these studies, mating a boar once every day for five or more consecutive days for a period of seven days.

Mating once a day for five consecutive days.—We tried to get as many boars as possible to mate daily for five consecutive days, but of the ones tried only those in Table 4 responded, and only two of these really fulfilled the schedule of mating. Probably boars 2485/27 and 2486/28 would have responded well too, but we could not try them because they were on a heavier mating schedule.

TABLE 4.—*Boars mated once a day for five consecutive days.*

Boar No.	June 21.			June 22.			June 23.		
	Volume of semen.	Spermatozoa per cmm.	Total spermatozoa.	Volume of semen.	Spermatozoa per cmm.	Total spermatozoa.	Volume of semen.	Spermatozoa per cmm.	Total spermatozoa.
	cc.		$\times 10^{10}$	*cc.*		$\times 10^{10}$	*cc.*		$\times 10^{10}$
2805/330	------	[a]	--------	160	258,000	4.13	160	360,000	[b] 5.76
2695/178	------	[a]	--------	289	[b]	--------	315	444,000	13.98
2615/91	340	364,800	12.4	180	382,000	6.87	------	[c]	--------
2564/34	------	[a]	--------	140	1,004,000	14.06	120	506,000	6.08

Boar No.	June 24.			June 25.			June 26.		
	Volume of semen.	Spermatozoa per cmm.	Total spermatozoa.	Volume of semen.	Spermatozoa per cmm.	Total spermatozoa.	Volume of semen.	Spermatozoa per cmm.	Total spermatozoa.
	cc.		$\times 10^{10}$	*cc.*		$\times 10^{10}$	*cc.*		$\times 10^{10}$
2805/330	180	140,000	2.59	200	259,000	5.18	245	138,000	3.38
2695/178	140	420,000	5.88	------	[c]	--------	------	[c]	--------
2615/91	265	244,000	6.46	175	155,000	2.71	------	[c]	--------
2564/34	140	346,000	4.84	187	278,800	5.21	180	163,000	2.94

[a] Not mated. [b] Few spermatozoa; alkaline reaction. [c] Did not mate.

Table 4 shows that the boars gave great numbers of spermatozoa almost invariably on the first day of the mating period, and that the numbers fell off in the course of the period. Boar 2564/34 on the first day gave 14.06×10^{10} spermatozoa, a very high number. On the second day the number was less than 50 per cent of that given on the preceding day, being 6.08×10^{10}. On the third day there was a further drop, and although there was a little rise on the fourth day, there was a very decided drop on the fifth day, the number being only 2.94×10^{10}, one-fifth the number given on the first day. With boar 2615/91 the results were the same. June 21 he gave 12.4×10^{10} spermatozoa; the next day 6.87×10^{10}; after a day of rest—on the 23rd he failed to mate—he gave again 6.46×10^{10} on the 24th; and lastly, on the 25th, he gave only 2.71×10^{10}. The 10-month-old boar 2805/330, on the other hand, gave a slightly different performance. The total number of spermatozoa given by him was well maintained. On the first day he gave only 4.13×10^{10}, considerably less than the average of normally mated boars, but there was a rise on the second day, then a bad drop on the third day, another rise on the fourth day, and another drop on the fifth day. On the whole, one can say that the tendency is for the number of spermatozoa to drop in the course of the period of daily mating.

TABLE 5.—*Boars double mated every other day.*

Boar No.	Time of day.	July 2.			July 3.			July 4.		
		Volume of semen.	Spermatozoa.		Volume of semen.	Spermatozoa.		Volume of semen.	Spermatozoa.	
			In cmm.	Total.		In cmm.	Total.		In cmm.	Total.
		cc.		× *10* [10]	*cc.*		× *10* [10]	*cc.*		× *10* [10]
2485/27	a. m.	230	351,000	8.08	-------	(b)	-------	160	163,400	2.61
	p. m.	120	122,500	1.47	-------		-------	145	85,400	1.23
2486/28	a. m.	260	298,000	7.75	-------	(b)	-------	220	138,600	3.05
	p. m.	150	175,800	2.64	-------		-------	240	95,200	1.40
2196/87	a. m.	-------	(a)	-------	280	(c)	-------	-------	(b)	-------
	p. m.	-------	-------	-------	160	56,800	0.91	-------	-------	-------
1883/284	a. m.	-------	(a)	-------	249	400,000	9.93	-------	(b)	-------
	p. m.	-------	-------	-------	-------	(c)	-------	-------	-------	-------
2200/91	a. m.	-------	(a)	-------	420	43,600	1.83	-------	(b)	-------
	p. m.	-------	-------	-------	-------	(c)	-------	-------	-------	-------

Boar No.		July 5.			July 6.			July 7.			July 8.		
		Volume of semen.	Spermatozoa.		Volume of semen.	Spermatozoa.		Volume of semen.	Spermatozoa.		Volume of semen.	Spermatozoa.	
			In cmm.	Total.		In cmm.	Total.		In cmm.	Total.		In cmm.	Total.
		cc.		$\times 10^{10}$	*cc.*		$\times 10^{10}$	*cc.*		$\times 10^{10}$	*cc.*		$\times 10^{10}$
2485/27	a. m.				170	73,000	1.24				170	151,000	2.56
	p. m.				105	,000	0.89				135	130,000	1.76
2486/28	a. m.				210	65,000	1.36				130	145,000	1.85
	p. m.				240	41,000	0.96				80	6,200	0.005
2196/87	a. m.	395	219,000	8.65		(b)		370	37,200	1.37			
	p. m.	360	35,000	1.26					(d)				
1883/284	a. m.	210	211,000	4.43		(b)			(d)				
	p. m.	230	221,000	5.08									
2200/91	a. m.	360	92,000	2.39		(b)			(d)				
	p. m.		(d)										

[a] Not mated. [b] Rest. [c] No spermatozoa; alkaline secretion. [d] Failed to mate.

Besides this reduction of the number of spermatozoa, which may be utilized as an index of sexual activity, there is a corresponding diminution in the eagerness of the boar to mate. These two facts lead to the belief that it is not wise to mate a boar once every day for an extended length of time. On the basis of these data it would seem that it is probably not wise to mate a boar once a day more than two or at most three days consecutively.

Mating twice a day every other day.—Mating twice a day every other day is quite a heavy program—too heavy for most of the boars studied. Only two boars, 2485/27 and 2486/28, were able to stand it. They mated eight times in seven days. This is a much heavier mating schedule than once a day for five days, consecutively; for although in the former there is an intervening day of rest, the rate of doing work, when work is done, is much heavier than in the latter. When a boar is mated twice a day, he does within twelve hours the work that another that mates only once a day, does in thirty-six hours. It follows from a well-known physiological principle that, doing work at a more rapid rate, the former boar became fatigued more rapidly and took a relatively longer time for recovery than the latter, working at a slower rate.

In Table 5 a great difference is recorded between the total number of spermatozoa contained in the first and second samples of semen given on the first day of double mating. In the case of boar 2485/27, the first sample of semen contained 8.08×10^{10} spermatozoa, and the second sample only 1.47×10^{10}. Boar 2486/28, similarly, gave in the first sample 7.75×10^{10}, and in the second sample only 2.64×10^{10}. July 4 boar 2485/27 gave only 2.61×10^{10} and 1.23×10^{10} in the first and second samples, respectively. As the period of double mating progressed, the samples of semen given by 2486/28 contained less and less spermatozoa. The same is true of 2485/27, except for a rise on the last day of the period. The number of spermatozoa in the second sample of semen was almost invariably much less than the number in the first sample; the exception was the second sample of semen from boar 1883/284, which contained a little more spermatozoa than the first sample of the same day, July 5.

The number of spermatozoa in the semen from the first mating after the intervening day of rest remained quite low, far below the arithmetic average given by the lightly mated boars, below also the twenty-four-hour production of the boars mated

once daily. Even if we added the spermatozoa from the first and second samples of semen collected July 4, they would not be sufficient to equal the number in the first sample of semen collected July 2. The same is true of the rest of the samples. Furthermore, we see that the double mating fails to produce as much spermatozoa as one daily mating. It seems then that the double sexual stimulus in twelve hours so strains the sexual organs that it takes the animal a relatively much longer time to recover his powers of ejecting mature spermatozoa.

DISCUSSION OF INTENSIVE MATING

The number of spermatozoa.—In all the foregoing the only criterion has been the number of spermatozoa ejected in each mating. Obviously, this criterion is far from being complete or adequate, for it takes into account only one of the products of sexual activity. The lack of an adequate criterion itself speaks eloquently of the general ignorance about the biology of the boar. That adequate criterion is yet to evolve from future work, and herein are indicated some of the things to be studied in order that it may eventually be fully worked out.

For the time being, at least, the preliminary use of the total number of spermatozoa as a criterion for judging the effects of intensive mating upon the boar is justified to this extent: in intensive mating the testes are the most important organs responding directly to the stimulus of mating, and the number of spermatozoa is the product of the response. One way of studying the effect of intensive mating consists in determining the number of spermatozoa ejected during intensive mating and comparing the number with the average number given during light mating.

By using this criterion in examining the data on intensive mating some light may be shed on a few of the "don'ts" in the use of the boar. Only in the most exceptional cases may it be considered wise to mate a boar twice a day. In the first place, the number of spermatozoa given in the second sample of semen is far below the average given by lightly mated boars, and meagerness in the number of the spermatozoa is highly undesirable. As pointed out, the probability that enough spermatozoa for fertilization would survive the action of the leucocytes in the uterus is much smaller when the spermatozoa are few than when they are many. In the second place, the second mating apparently so strains the sexual organs that a much

longer period of rest is afterwards needed by the boar for sexual recuperation.

For the same reason it is not wise to mate a boar once every day for more than two days in succession, for even by the third day the number of spermatozoa falls far below the average number given in a light mating.

The question arises, why does the second mating in a day give only a very meager amount of spermatozoa; the epididymes apparently contain more spermatozoa than can be ejected in several ejections. It would seem as if the spermatozoa required a certain minimum length of time outside the testes, in the epididymes, to be transformed from the nonmotile type that they are when they get out of the testes into the epididymes to the motile types found at the caudal portion of the epididymes.

SOME BIOLOGICAL FACTORS THAT INFLUENCE MATING

Assuming that the conformation of the boar is not abnormal, the following factors must be considered important in influencing the mating of the boar:

The amount of sexual secretion.—This factor is important, because the amount of energy consumed in the production of a secretion is at least directly proportional to the amount of the given secretion; that is, the greater the amount of the secretion the greater the amount of energy spent in producing it. It has been pointed out that in a light mating schedule the average volume of the semen is 252.8 cc. In some cases that volume reached 500 cc, and in one case it reached 540 cc. Such a voluminous secretion tends to upset some physiological balance, especially if the mating is done often.

To examine the reasons for this assertion, the sexual secretion may be divided into three main constituents: (*a*) the spermatozoa, (*b*) the nonliving organic matter in suspension (proteins, carbohydrates, and lipoids), and (*c*) the mineral matter.

The spermatozoa.—Table 4 shows that some boars are able to eject as many as 5.18×10^{10} spermatozoa within twenty-four hours, provided the daily matings are not prolonged. Lightly mated boars give an average of 7.83×10^{10} spermatozoa, and the number may be as high as 16.34×10^{10}. The production of such a great number of spermatozoa, which means the elaboration of a relatively large amount of complex substances, like the nucleo-proteins, undoubtedly uses up a great deal of energy.

The importance of the number of spermatozoa becomes somewhat clearer if a comparison be made between the total number

of spermatozoa ejected by the boar and that by the ram. The ram's semen is very dense with spermatozoa, there being about 2.0×10^9 per cubic centimeter, or a total of 2.0×10^9 to 4.5×10^9 in 1 cc to 1.5 cc, which is the normal volume of the ram's semen.[2] The average number of spermatozoa in the boar's semen is 7.83×10^{10} or 78.3×10^9. The boar's semen, therefore, contains about 19 times as many spermatozoa as the semen of the ram—assuming 3.75×10^9 as the average number of spermatozoa in the ram's semen. The ram, then, would have to mate 19 times in order to produce the number of spermatozoa given by the boar in one ejection—that is, assuming that the ram gives 3.75×10^9 spermatozoa in each successive ejection, which is highly improbable.

The great number of spermatozoa ejected, the production of which takes a great amount of energy, is one of the important reasons why a boar cannot mate normally more than twice a day at most.

The organic substances in the semen.—If it is assumed that the amount of organic substance per cubic centimeter of semen does not vary much, it would follow that the greater the volume of the semen the greater the amount of organic substances; there would, in fact, be a linear relation between the volume of semen and the amount of organic substances. The importance of the organic substances is evidently in the amount of energy the boar has to spend in elaborating them; the more organic substances there are, the greater the amount of energy needed to produce them, and the more easily the boar gets exhausted. Among the domestic animals the boar gives the greatest volume of semen, and probably secretes the greatest amount of organic substances in the semen.

The inorganic substances in the semen.—It is probable that the amount of salts in the semen varies little, for if it varied much the osmotic pressure would likewise vary, and this would kill the spermatozoa. In man and the stallion, Koltzoff points out that the semen contains about 0.9 per cent of salts. If the semen of the boar contained about the same concentration of salts, the average volume of semen given by a lightly mated boar, 252.8 cc, would contain 2.3 grams of salts, and a 500-cc sample 4.5 grams.

[2] The information on the average number of spermatozoa in the semen of the ram was furnished the author by Mr. V. K. Milovanoff, of the Institute of General Animal Husbandry, Moscow.

Two and three-tenths grams of minerals by itself is not much, but when this amount is compared with the quantity that is probably present in the semen of the ram, the horse, or the bull, it is large. Assuming that the semen of all these animals contains 0.9 per cent of salts, the ram would give only 0.0135 gram in 1.5 cc of semen; the bull, 0.0405 in 4.5 cc (the average volume of a bull's semen); and the horse, 0.45 gram in 50 cc (the average volume of the stallion's semen).[3]

It is clear, that the relatively large amount of salts in the semen of the boar probably influences his mineral metabolism. Mating probably upsets the mineral balance in the boar more than in the males of any other species of domesticated animal. This is probably especially true in the case of an intensively mated boar. Therefore, the study of this factor should prove interesting and profitable.

The amount of energy needed for mating.—It takes the boar from five to eight minutes to complete the sexual act. This is from the time the penis is inserted into the vagina to the time the boar is ready to dismount. The ram and the bull complete the sexual act almost instantaneously with the insertion of the penis into the vagina. The stallion takes about two to three minutes to complete the act. Apparently, in animals where the development of the sexual organs is comparable, the length of time taken to complete the sexual act is a function of the amount of the semen given. The boar, giving the largest amount of semen, takes the longest time to complete the act; the stallion, ejecting the next largest amount of semen, completes the sexual act in the next longest time; the bull and the ram ejecting very little semen complete the act in a very short time.

The length of time required to complete the act can, in a way, show how much muscular and nervous energy are spent in mating. The longer it takes to complete the act, the greater the relative amount of energy consumed. Thus, the ram would consume the least relative amount of energy in mating, and the boar the largest relative amount. This is another reason for the boar's inability to mate as heavily as the ram, for instance.

SUMMARY

1. The semen from the first mating of the boar does not as a rule give spermatozoa different from those given in subsequent

[3] The information on the relative volume of the semen of the bull, the ram, and the stallion was given to me by Mr. V. K. Milovanoff, of the Institute of General Animal Husbandry, Moscow.

matings. There were two boars, however, that gave repeatedly dead spermatozoa. It was attempted provisionally to explain this phenomenon as a case of abnormal resorption of spermatozoa and the consequent formation of antibodies against the spermatozoa.

2. The total number of spermatozoa ejected by a boar, especially in some cases, is quite variable. By considering a number of boars together, however, the average number of spermatozoa in a normal ejection can be determined. That average is 7.83×10^{10}.

3. The total number of spermatozoa given is a measure of the fecundity of the boar. This is an index of a boar's quality which may be of practical value in selection work. It was pointed out that poor fecundity probably means poor fertility.

4. There is no correlation between the volume of the semen and the total number of spermatozoa.

5. The volume of the semen as well as the density of the spermatozoa and the total number varies widely, even in the same individual.

6. From the data, it would appear that it is not wise to breed a boar until he is about 14 months old, and then only lightly. It is probably best not to mate boars intensively until they get to be around 2 years old or more.

7. It would seem as if a boar should not be mated more than once a day under ordinary conditions. Our data suggests also that a boar should probably not be mated any more than once a day for two consecutive days. And after such a period, he should be given a rest of at least two days.

8. The factors that prevent the use of the boar twice a day or more have been discussed. Fully to establish the points brought out, however, requires experimental procedure.

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ILLUSTRATION

TEXT FIG. 1. Longitudinal section of artificial vagina.

PLATE 1.

A NEW PHILIPPINE PHALLOID (ANTHURUS BROWNII)

By José Miguel Mendoza

Mycologist, National Museum Division, Bureau of Science, Manila

ONE PLATE

The Philippines are rich in phalloids, many of them interesting even to the layman. Some of the phalloids are beautiful, others have queer shapes, while from a few emanates an offensive fetid odor which can be detected from a considerable distance. While the group is well known to science, unfortunately few species of *Anthurus* have been studied critically. This is the first time this genus is reported from the Philippines. The new form herein reported and described is based on study of fresh specimens.

ANTHURUS BROWNII Mendoza sp. nov. Plate 1, figs. 1 to 5.

Peridium obovatum, albi coloris, circa 5 cm. in diametro, asperum, ex tribus stratis compositum quorum externum tenueque est furfuraceum, illud vero internum et crassum, gelatinosum; receptaculo stipitato, cavo, brevi, cylindraceo, longo circa 5 cm., 4 cm. in diametro, albi coloris, tenui, in brachia 6 ad 9 scisso, primo ad apiceum conjuncto, posterius vero tempore separato; superficie interna brachiorum gleba fœtida atraque ornata; sporidiis, 3.19 micra ad 4.46 micra longis, latis 2.87 micra ad 3.82 micra, congestis se tincta exhibentibus, sub microscopio vero viridis cinerei coloris.

Peridium obovate, white, about 5 cm in diameter, sometimes smaller, outer coating rough to the touch, composed of 3 distinct layers, the other being thin and furfuraceous, the inner thick and gelatinous. Receptacle stipitate, short, cylindrical, hollow, up to 5 cm long, about 4 cm in diameter, rather thin, white throughout, broader at the top than below, divided into 6 to 9 arms; arms white, 3.5 to 6 cm long, finely wrinkled on the interior, longitudinally sutured at the outside, when young the arms are united, but soon break off at the apex, several fingers are bifurcated at the extremities. Gleba borne on the inner surface of the arms, extremely fetid, black; spores are of a tinted color

when in mass, olive-gray under microscope, oval to elliptical, 3.19 to 4.46 μ long, 2.81 to 3.82 μ broad.

LUZON, Rizal Province, Muntinlupa, *Bur. Sci. 55052, E. D. Gutierrez* (type), October 15, 1932, on sandy shore of Laguna de Bay, *Bur. Sci. 55625, J. M. Mendoza,* July 20, 1933, on sandy shore, near Laguna de Bay, *Bur. Sci. 55626, J. M. Mendoza,* November 12, 1932, on sandy shore, Laguna de Bay, *Bur. Sci. 55627, J. M. Mendoza,* July 15, 1933, on sandy shore of Laguna de Bay, *Bur. Sci. 55628, J. M. Mendoza,* August 20, 1933, *Bur. Sci. 55629, J. M. Mendoza,* October 1, 1932, *Bur. Sci. 55630, J. M. Mendoza,* July 10, 1933, on sandy soil; *Bur. Sci. 55631, J. M. Mendoza,* October 25, 1933, on sandy soil.

Anthurus brownii resembles *A. archeri* in many respects, differing, however, in color which is white while that of *A. archeri* is red, and in having six to nine arms. *Anthurus archeri* has five to seven arms.

The species grows in sandy soil, mixed with decayed organic matter, and is found from June to about November.

The author dedicates the species to Dr. W. H. Brown, director of the Bureau of Science, for his many years of botanical work in the Philippines.

ACKNOWLEDGMENT

The writer is indebted to Dr. E. Quisumbing, botanist, National Museum Division, Bureau of Science, for criticisms.

ILLUSTRATION

PLATE 1. ANTHURUS BROWNII SP. NOV.

FIG. 1. The phalloid in unexpanded stage; × 0.75.
2. Young stage showing the united arms; × 0.75.
3. A full-grown fungus; × 0.75.
4. The same after removing the volva; × 0.75.
5. The fungus in its natural habitat. (Photographed by Domingo Farol, of the Bureau of Science.)

ILLUSTRATIONS

PLATE 1. LEPIOTA AMERICANA PECK

FIG. 1. The mushroom in natural habitat, showing different stages. (Photographed by Domingo Farol of the Bureau of Science.)

2. The mushroom as sorted ready for the market. (Photographed by Domingo Farol of the Bureau of Science.)

PLATE 2. LEPIOTA AMERICANA PECK

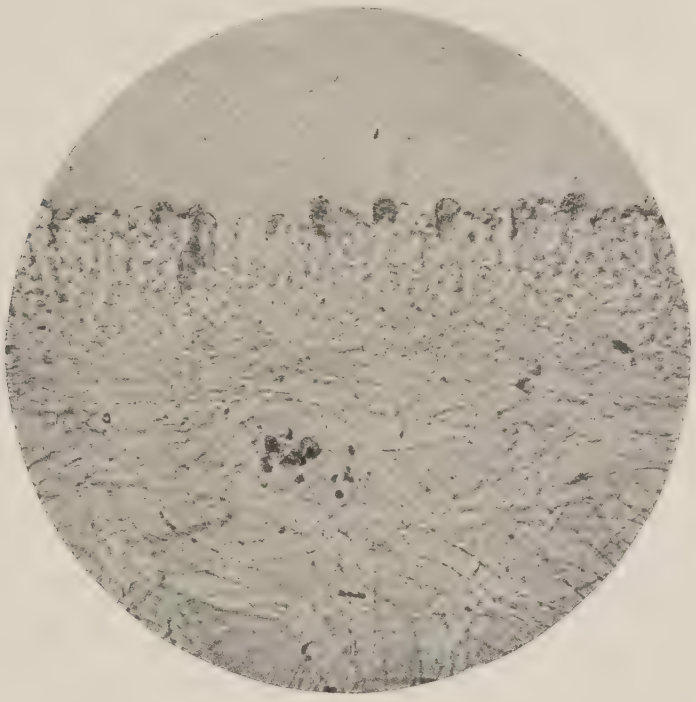
FIG. 1. Photomicrograph of a section of the gill, showing the hymenium with basidia; × 235.

2. Photomicrograph of the spores, from spore collection; × 433.

1

2

PLATE 1.



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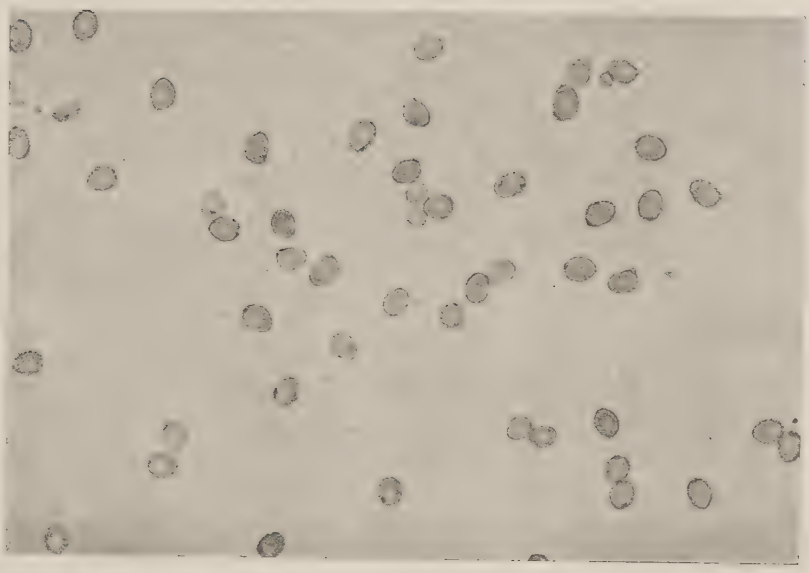

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PLATE 2.

THE DISTRIBUTION OF BANANA WILT

By Otto A. Reinking

FIVE PLATES

INTRODUCTION

During the period 1925 to 1927, while the writer was making a study of the various banana varieties in the world, an opportunity presented itself to study the distribution of banana wilt (Panama disease) caused by *Fusarium cubense*. Former investigations relative to distribution indicated that the disease was world-wide, but authentic determination of the causal organism had not been made in all cases. The present studies were carefully checked with isolation and fungus determinations based on recent reports.(9, 11, 16) Up to the time of this investigation the disease had been recorded, outside of the Caribbean region, from Hawaii by Higgins,(7) Carpenter,(5) and Wilcox;(15) from the Philippines by Lee and Serrano;(8) from India by Basu,(1) Butler,(3) and Shaw;(12) from Australia by Tryon;(13) and from the Canary Islands and Africa by Dunlap through the Imperial Bureau of Mycology.(18, 19)

SCOPE OF PRESENT STUDIES

Investigations on banana wilt were made by the writer in the following countries: Philippine Islands, French Indo-China, Straits Settlements (Singapore and Penang), Federated Malay States, Siam, Dutch East Indies, Australia, Burma, and India. The disease was observed in all of these countries except French Indo-China. It is highly probable that it is present in the latter country, although it was not encountered during the survey. Special studies verified its presence in the localities given in Table 1.

DETAIL OF INVESTIGATIONS

A detailed account of the investigations, according to the countries where the disease was found, the varieties affected, and the studies made of it, is presented in the following discussion.

TABLE 1.—*Banana wilt (Panama disease); geographic distribution of diseased plants studied.*

Country.	Locality.	Date of observation.	Culture studies.
Philippine Islands	Los Baños, Laguna	Mar., 1925	*F. cubense* isolated.
Straits Settlements	Singapore	Oct., 1925	Do.
Do	Penang	Dec., 1925	No isolation.
Federated Malay States	Serdang	Dec., 1925	*F. cubense* isolated.
Do	Kuala Lumpur	Dec., 1925	No isolation.
Do	Kajang	Dec., 1925	Do.
Siam	Bangkok	Nov., 1925	*F. cubense* isolated.
Dutch East Indies	Buitenzorg, Java	Mar., 1926	No isolation.
Do	Pasar Minggoe, Java	Apr., 1926	*F. cubense* isolated.
Do	Tohoedan Experiment Station, Solo, Java.	Apr., 1926	No isolation.
Do	Pasoeroean, Java	Apr., 1926	Do.
Do	Banda, Molucca Islands	Aug., 1926	Do.
Australia	Brisbane (Pinkenba)	Oct., 1926	*F. cubense* isolated.
Burma	Hmawbi Experiment Gardens, near Rangoon.	Dec., 1926	No isolation.
India	Poona, Bombay Presidency	Jan., 1927	*F. cubense* isolated.
Do	Munsiganj, Dacca, Bengal	Jan., 1927	Do.
Do	Rampal, Dacca, Bengal	Jan., 1927	Do.

PHILIPPINE ISLANDS

The disease was first noted by Lee and Serrano(8) as being severe on the Latundan variety. Culture studies made by the writer also proved the disease to be due to *Fusarium cubense.*

BANANA VARIETY AFFECTED

1. Latundan

During March, 1925, studies of the disease situation were made by the writer. The Latundan variety was the only one observed to be affected. This banana is a common type with a world-wide distribution. In the West Indies a type of the variety is commonly known as the Apple or Manzana banana. In the Philippines this banana is most commonly planted for commercial purposes. The disease was observed only in the Manila region, indicating that it may have been introduced into the Islands. It was observed in its characteristic form in Calamba, Pansol, Lalakay, Los Baños, and Calauan, all in Laguna Province, Luzon. In some plantings 100 per cent infection was noted.

The characteristic wilting and yellowing of leaves along with severe splitting of the pseudostem characterized the disease (Plate 1, fig. 1). Interior symptoms, including the typically discolored vascular bundles, were present in each case. Cul-

ture studies showed that in all cases of disease typical *Fusarium cubense* was present in a pure state, thereby corroborating the finding of Lee and Serrano.

STRAITS SETTLEMENTS AND FEDERATED MALAY STATES

Up to the time of this investigation the banana-wilt disease had never been reported from these regions. Studies made from October to December, 1925, showed the disease to be present in the Straits Settlements on Singapore Island (10) and in the environs of Penang, and in the Federated Malay States at Kuala Lumpur, Serdang, and Kajang. It undoubtedly is widespread throughout the Federated Malay States wherever the susceptible varieties are planted. Recent studies made by Ward (14) apparently confirm these findings.

BANANA VARIETIES AFFECTED

1. Awak; Penang.
2. Embun; Singapore and Kajang.
3. Rastali; Penang, Kuala Lumpur, and Serdang.

The Embun variety is commonly and severely attacked (Plate 1, fig. 2; Plate 2, fig. 1). This banana is the common Gros Michel of commerce universally planted in the West Indies. Pisang rastali is similar to the Latundan variety of the Philippine Islands and the Apple or Manzana variety of the West Indies. The Awak variety is similar to the Nam Wa of Siam. It is commonly cultivated in Penang and in various parts of the Federated Malay States. External and internal symptoms of disease on all varieties were typical of the true banana wilt of the Caribbean region. Isolations made from specimens collected at Serdang and Singapore resulted in pure cultures of *Fusarium cubense*. In no cases of typical disease were other species of fusaria isolated.

SIAM

Banana wilt never had been reported as occurring in Siam. Observations made in November, 1925, showed the disease to be widespread and severe on one variety grown about Bangkok.

BANANA VARIETY AFFECTED

1. Nam Wa.

The Nam Wa variety is the commonest on the market and in the field. It is a starchy type that is usually cooked. The disease shows typical symptoms of wilting, but splitting of the pseudostem was not so common (Plate 2, fig. 2). Frequently plants appear to be partially resistant and trying to throw off

the trouble. The disease was found on a trip into the Bang Sorn district and at the Bangkok Noi Experimental Orchard just out of Bangkok. Specimens were collected and taken to the Pasteur Institute for examination and culture studies. Microscopic examination showed the typical discolored vascular bundles and mycelium in the xylem tissue. Isolation from diseased portions produced pure cultures of *Fusarium cubense*.

DUTCH EAST INDIES

A special investigation of banana wilt was made in Java because of previous studies conducted by Gaumann(6) on a widespread vascular disease of bananas attributed to bacteria. Gaumann, in his study of this disease, failed to isolate *Fusarium cubense*. He isolated six other species of fusaria from old diseased material, but none of these produced disease in inoculation tests. At no time was one predominating fusarium found. Gaumann concludes that the vascular disease of the bananas in Java was caused by *Pseudomonas musæ* and that the genuine destructive Panama disease is similar but distinguished by the presence of *F. cubense* in the secondary vascular bundles.

In the recent study of the vascular diseases of banana in Java made by the writer the typical banana wilt (Panama disease) caused by *Fusarium cubense* was observed. External and internal symptoms were typical of those described by Brandes(2) in the West Indies. *Fusarium cubense* was isolated in pure culture from all typical cases of disease. Many banana varieties were found to be susceptible to disease, while on the other hand many resistant varieties exist.

BANANA VARIETIES AFFECTED

Pasar Minggoe, Java. Ragoenan Agricultural Experimental Station (near Batavia).

1. Ambon bodas.
2. Ambon poetih (Gros Michel) (Plate 3, fig. 1).
3. Besi.
4. Boeboejan.
5. Kepok koening.
6. Kloetoek soekoen.
7. Kool.
8. Mentek.
9. Poelo alar.
10. Radja.
11. Radja djawa.
12. Radja koesta.
13. Radja pakoewon (Plate 3, fig. 4).
14. Radja pelilit.
15. Radja pretel.
16. Radja sereh bodas (Latundan, Philippines).
17. Radja sijem (Nam Wa, Siam).
18. Sembot.
19. Silo Kapoek.
20. Soesoe (Plate 3, fig. 3).
21. Songgroito (Sri) (Plate 3, fig. 2).
22. Sribali (Gros Michel type).
23. Tjandi.
24. Tjeleket.
25. Tjoemet.

Buitenzorg, Java. Tjipakoe Experiment Station.

1. Radja.

Solo, Java. Tohoedan Experimental Station.

1. Galigis.
2. Kapas idjo.
3. Kepok ambon.
4. Panggra.
5. Sobo kepok.
6. Sobo madoera.
7. Soesoe.
8. Soesoe bodas (Latundan, Philippines).

Pasoeroean, Java. Pasoeroean Experimental Station.

1. Gading.
2. Gembang.
3. Kapas idjo.
4. Panggra.
5. Radja djawa.

Moluccas. Banda Islands (Banda Neira and Poeloe Ay).

1. Radja (Ambon poetih) (Gros Michel).
2. Soesoe (Latundan, Philippines).

Possibly some of the local variety names in the above list refer to the same banana. It was impossible to check each definitely because of lack of fruit. The same banana names used in different localities of Java cannot always be taken as referring to one distinct variety, because the same banana variety receives different names depending on the locality from which it has been collected. The Gros Michel banana of the West Indies is called Ambon poetih in various parts of Java and Radja in Amboina and the Banda Islands. The Radja variety in Java is therefore totally different from the Radja of Ambon. The name Soesoe as used in Java may refer to two different varieties of banana. In one locality the common Apple banana of the West Indies is called Radja sereh bodas, in another locality it is called Soesoe or Soesoe bodas. The name Soesoe is also given to an entirely different banana similar to the real Soesoe in the Federated Malay States. These few cases are cited to make it clear that local names, as often given to bananas in the Dutch East Indies, may be very misleading. The names given in the list above have been taken directly from the experimental station records cited or from the local names according to the place listed. Consequently a duplication of actual varieties may exist in the list, as fruit was not always present for differentiation purposes.

A careful study made in March, 1926, for disease in the banana collection at the Ragoenan Experimental Station, situated at Pasar Minggoe, Java, showed the disease to be spread throughout the entire collection. According to the list given

above, twenty-five varieties among some one hundred fifty types were found to have the disease. During the following month a survey of the collection at the Tohoedan Experimental Station at Solo showed eight varieties affected, and at the Pasoeroean Experimental Station at Pasoeroean in eastern Java, five varieties were affected. The banana wilt is evidently widely spread throughout the Dutch East Indies, as typical cases of diseases were found on Banda Neira and Poeloe Ay of the Banda Islands in the Molucca group.

SEVERITY OF DISEASE IN JAVA

The banana wilt in Java, as well as generally throughout the Far East, is usually not much in evidence in the small native plantings, because some five to twenty varieties of bananas are commonly planted in the same plot. Most, or a great part, of these varieties are resistant, and one occasional sick plant of a susceptible variety causes little anxiety and is often not recognized as suffering from an infectious trouble. The Ambon poetih (Gros Michel) is possibly the most susceptible of all varieties. It is seldom extensively planted by the natives as they prefer other kinds for their own use. Only when this variety is extensively planted, as is done near a special market, does the disease become really noticeable. Solid plantings of Ambon poetih on diseased soil may show severe disease within six months and may have as high as 90 per cent disease after twelve months of growth. The following diagram illustrates severe infection of Gros Michel in small, solid plantings made at the Ragoenan Agricultural Experimental Station at Pasar Minggoe, Java. The O's and and X's designate the various plants according to the planting plan. X's indicate locations of diseased plants with external symptoms.

Banana wilt in 11.5-month-old planting at Pasar Minggoe, Java.

[O=healthy plant; X=diseased plant.]

Variety, Ambon Poetih.	*Variety, Sribali.*
X X O O X O O	O X X O X O X
X X X X X X X	O O X O X O X
X X X X O X X	O X X O X X
X O O O O X X	O X X O X O
Total plants, 28.	Total plants, 26.
Diseased plants, 19.	Diseased plants, 14.
Diseased, 68 per cent.	Diseased, 54 per cent.

The variety Ambon poetih is the Gros Michel of the West Indies. Sribali was reported as being a type of Ambon poetih. The plants listed above were planted April 15, 1925. March 4, 1926, eleven and one-half months after planting, the Ambon poetih variety had 68 per cent disease (Plate 3, fig. 1; Plate 5, fig. 1) and both varieties were 100 per cent diseased. The plants were small as they were growing on rather heavy soil. The photographs clearly show the external symptoms of disease identical to that of the banana wilt in the West Indies.

Examination made in March, 1926, of other small plantings of single varieties showed severe infection in some cases. A list of these varieties with percentage of infection is given below:

Banana wilt in plantings at Pasar Minggoe, Java.

	Infection. Per cent.
Radja sereh	40
Radja sereh bodas, 9 diseased among 98 plants	9
Radja sijem	90
Soesoe, 2 diseases among 64 plants (Plate 4, fig. 2)	3
Songgroito (Sri) (Plate 3, fig. 2)	10

Radja sereh is similar to the Latundan of the Philippines and the Apple or Manzana variety of the West Indies. Radja sereh bodas is a type of Radja sereh. Radja sijem is similar to the Nam Wa of Siam. These two varieties are commonly found diseased wherever grown. The disease is generally associated with definite varieties grown under different conditions in various parts of the world. The Soesoe variety is the Federated Malay States type. Songgroito (Sri) is a Dutch East Indies type that is frequently subject to disease.

Isolations were made of the first cases of disease observed. The external, internal, and microscopic aspects of the cases studied were identical with the real banana wilt (Panama disease) of the West Indies. Four typical cases of disease were selected for isolations as follows:

Variety.	Collected.	Locality.
Radja	Feb. 13, 1926	Buitenzorg (Tjipakoe), Java.
Ambon poetih	Feb. 13, 1926	Pasar Minggoe, Java.
Do	Feb. 13, 1926	Do.
Songgroito (Sri)	Feb. 13, 1926	Do.

In each case a pure culture of *Fusarium cubense* was isolated. No other fusaria were present in the diseased tissues. Deter-

minations of *Fusarium cubense* were made from cultural characters and spore measurements of the fungus growing upon potato agar and rice. The typical aromatic odor of the organism when grown on rice was produced.

SPECIAL ISOLATION STUDIES

In order to prove definitely that *Fusarium cubense* is associated with all the varieties of bananas subject to the disease, sixteen varieties found diseased were collected March 25, 1926, at the Ragoenan Experimental Station, Pasar Minggoe, and taken to Buitenzorg for isolation and culture studies. The writer is indebted to Dr. Van Hall and members of the Institute voor Plantenziekten at Buitenzorg for use of laboratory and equipment. Each variety collected had typical symptoms of the disease. A list of the varieties from which isolations were made follows:

Isolations of pure cultures of F. cubense, Pasar Minggoe banana collection, March, 1926.

Variety.	Place of isolation.
1. Ambon bodas	Pseudostem.
2. Besi	Do.
3. Kloetoek soekoen	Do.
4. Kool	Do.
5. Mentek	Do.
6. Radja (batoel)	Do.
7. Radja koesta	Do.
8. Radja pakoewon	Do.
9. Radja pelilit	Do.
10. Radja pelilit	Rhizome.
11. Radja pretel	Pseudostem.
12. Radja sereh bodas	Do.
13. Radja sijem	Do.
14. Sembot	Do.
15. Soesoe (Banjoewangi type)	Do.
16. Tjeleket	Do.

The Besi and Sembot varieties had only mild cases of infection.

Isolations were made March 26 on acidified potato-agar plate cultures by taking small pieces of infected pseudostem and plating them out. In one case, that of Radja pelilit, isolations were made from the infected vascular bundles of the rhizome. The portion of the diseased pseudostem of variety Tjeleket was taken near the top of the plant just below the leaf blades. In all other cases portions of the pseudostem, approximately one foot above the ground, were taken. Microscopic examinations of

diseased bundles showed in all cases the presence of mycelium in the xylem tubes.

March 29 examination of the plates showed that in each case a pure culture of fusarium grew from the diseased portions. The fusaria produced from each were so striking and pure that there was no question about the presence of one single strain in all varieties. The growth from infected portions of the rhizome of the Radja pelilit was identical with that from the pseudostem.

Transfers of the fungus developed from each variety were made to tubes of potato agar, rice, and stems of *Tephrosia candida,* for final identification of the organism. Subsequent examination of these cultures proved that the organism in every case was a pure culture of *Fusarium cubense* identical in every respect, physiologically and morphologically, with the organism as described from Central America and the West Indies.(11)

The isolations made from typical cases of banana wilt (Panama disease) from this region of Java proved that *Fusarium cubense* was present in a pure state in every variety from which isolations were made. In no case was any other type of fusarium isolated. There is no reason to believe that the disease in Java is in any way different from the disease in other parts of the world.

AUSTRALIA

Banana wilt undoubtedly has been present in Australia for many years. Tryon(13) in 1912 mentioned a disease investigated by Dr. Joseph Bancroft in 1874–1876 attacking the sugar banana in southern Queensland. The disease was also reported as occurring in the common plantain growing in that region. Tyron described the disease and stated that it was similar to the Panama disease. The description of the disease leaves no doubt that the trouble was identical with the banana wilt (Panama disease). This is especially true in view of the present findings.

The following banana varieties have been reported to be affected.

BANANA VARIETIES AFFECTED

1. Lady Finger.
2. Sugar banana.
3. Gros Michel.
4. Plantain (variety name not mentioned).

The writer made observation and isolation studies of the disease only on the Lady Finger variety. The disease was reported to me by Australian investigators as occurring on the

other named varieties. The Lady Finger banana is commonly grown in Australia. It should not be confused with the type designated as Lady Finger in the Caribbean region. The Australian Lady Finger banana is similar to the Rio banana of Tahiti and the Brazilian banana of Hawaii.

The disease was studied in a plantation at Pinkenba, down the river from Brisbane. It was severe on the Lady Finger variety and showed typical symptoms with split pseudostems and wilted, yellow leaves. Diseased specimens of the rhizome and pseudostem were taken to Brisbane for microscopic examination and isolation studies. The writer is indebted to Prof. E. J. Goddard, of Queensland University, for the use of laboratories and equipment. Internal symptoms were typical of the true banana wilt except that more yellowed strands predominated and more rotted areas were present throughout the infected pseudostem. Certain varieties of bananas affected with the fusarial wilt may show a preponderance of yellowed vascular bundles over the reddish or black. Pure cultures of *Fusarium cubense* were isolated from the rhizome and pseudostem. The fungus was isolated both from reddish black and yellow stages of discoloration in bundles.

BURMA

The banana wilt disease was observed in southern Burma near Rangoon at the Hmawbi Agricultural Experimental Station. The disease was not seen in the northern Mandalay region or in central Burma. Only one variety of banana was found to be attacked.

BANANA VARIETY AFFECTED

1. Kala.

The Kala variety is similar to the Latundan of the Philippines and the common Apple or Manzana banana of the West Indies. This variety and the Gros Michel are the two most commonly attacked in all parts of the world where grown. All characteristic symptoms including splitting were observed on diseased plants. Microscopic examination showed discolored vascular bundles with fungus mycelium in the xylem tubes. No isolations were made.

INDIA

Basu(1) in 1912 reported the presence of a banana disease in Chinsurah. Certain varieties were said to have been killed out entirely. A description of the disease leaves no doubt that the trouble was identical with the banana wilt (Panama disease). This is especially true in view of the present findings. In 1914 Shaw(12) and in 1915 Butler(3) again reported the presence of the disease.

The present investigation covered the Dacca District, Bengal, in eastern India, and the Poona District, Bombay, in western India. In the Dacca region it was severe, especially in garden plantings that had been cultivated for years about the farm houses. The disease was observed in the Munsiganj and Rampal areas of the Dacca region. At Poona the disease was observed only on a seedling variety found growing at the Ganeshkhind Botanical Gardens.

BANANA VARIETIES AFFECTED

1. Kabari; Dacca District, Bengal.
2. Sonkel Chanda (from seed); Poona District, Bombay.

The Kabari variety is similar to the Nam Wa of Siam and Awak of the Federated Malay States. It is commonly cultivated in the Far East. Sonkel Chanda is a seedling variety grown from the seed of the common Sonkel variety of India. Typical external symptoms of wilting and splitting were present in each case. Microscopic examination showed the presence of mycelium in the xylem tubes. Pure cultures of *Fusarium cubense* were isolated from each case of disease.

DISCUSSION

The foregoing studies on banana wilt (Panama disease) clearly show that this disease is generally widespread in most banana sections of the world. The disease is generally associated with definite varieties wherever grown and affects these varieties most severely. Gros Michel apparently is the most susceptible. The Apple Manzana variety as known in the West Indies is generally found to be diseased to a greater or lesser degree. The Awak variety of Penang or Nam Wa of Siam is commonly diseased where grown throughout the Far East. The

following table gives the local variety names in the various countries where these diseased varieties were observed:

Banana varieties most commonly found diseased.

Gros Michel; West Indies and Central America. Also known as—
Embun; Federated Malay States.
Ambon poetih; Java.
Manzana or Apple; West Indies and Central America. Also known as—
Latundan; Philippine Islands.
Rastali; Federated Malay States and India.
Kala; Burma.
Radja sereh; Java.
Awak; Penang, Straits Settlements. Also known as—
Nam Wa; Siam.
Radja sijem; Java.
Kabari; India.

Fusarium cubense was isolated from all typical cases of disease studied. No other fusarium was found in typical diseased lesions. Brandes(2) clearly proved that typical disease can be produced by inoculation with *Fusarium cubense*. Later studies by Carleton(4) and Reinking(9) also show this to be true. External and internal symptoms were identical in all cases of disease observed on the various varieties as grown in all countries visited. The universal presence of *Fusarium cubense* in typical cases of banana wilt throughout the areas covered in this study, considered with the successful inoculation studies previously made and the similarity of external and internal symptoms, seem to be evidence enough that the disease is everywhere identical.

SUMMARY

Symptomatic and isolation studies clearly show that the banana wilt (Panama disease) is identical in the following countries: Philippine Islands, Straits Settlements (Singapore, Penang), Federated Malay States, Siam, Dutch East Indies, Austrialia, Burma, and India.

The banana wilt (Panama disease) is world-wide in its distribution, being found in practically all banana regions.

Certain varieties of bananas are highly susceptible to the disease wherever grown.

The Gros Michel is subject to the disease in most parts of the world where it is grown.

Studies conducted in Java show the presence of *Fusarium cubense* in all typical cases of infection. No other fusaria were found associated with typical cases of the disease.

There is no reason to believe that the disease in Java is in any way different from the disease in other parts of the world.

The universal presence of *Fusarium cubense* in typical cases of banana wilt (Panama disease) throughout the areas covered in this study, considered along with the successful inoculation studies previously made and the similarity of external and internal symptoms, seem to be evidence enough that the disease is everywhere identical.

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